# PHARMACOLOGICAL EVALUATION OF MARINE ALGAE OF MANDAPAM COAST: *ULVA RETICULATA*

***A Dissertation submitted to***

**THE TAMIL NADU DR. M. G. R. MEDICAL UNIVERSITY**

**CHENNAI - 600 032.**

**In partial fulfillment of the requirements for the award of the Degree of**

**MASTER OF PHARMACY**

**IN**

**BRANCH-IV PHARMACOLOGY**


**Submitted by**

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This is to certify that the dissertation entitled **"PHARMACOLOGICAL EVALUATION OF MARINE ALGAE OF MANDAPAM COAST: *ULVA RETICULATA*"** submitted by **Mr. D. KALIVARATHAN [Reg. No: 261525151]** for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by him in the Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli under my direct guidance and supervision.

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This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in Pharmacology, of The Tamil Nadu Dr. M.G.R. Medical University, during october 2017

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## ACKNOWLEDGEMENT

I am very much indebted to say that this project was the result of the help and encouragement of many people, who contributed directly or indirectly towards the successful submission.

I admit and acknowledge with sincere heart for the guidance and encouragement received from my guide **Dr. S. Karpagam Kumara Sundari, M.Pharm, Ph.D., Head, Department of Pharmacology,** Periyar College of Pharmaceutical Sciences, Tiruchirappalli**.** I also express my sincere thanks to my guide for her involvement, guidance, support, encouragement and gave me valuable suggestions to complete this project successfully. Her guidance helped me at all the times of research and writing the thesis. I have been extremely happy to have a guide who cared so much about my work.

I am indebted to acknowledge and heartful thanks to our Principal **Prof. Dr. R. Senthamarai, M.Pharm, Ph.D.,** Periyar College of Pharmaceutical Sciences, Tiruchirappalli for providing necessary infrastructure and constant support throughout my research work.

I extend my warm thanks to the **Prof. Dr. A.M. Ismail, M.Pharm, Ph.D.,** Professor Emeritus, Department of Pharmacy Practice and **Prof. Dr. G. Krishnamoorthy, B.Sc, M.Pharm, Ph.D.,** Vice-Principal & Head, Department of Pharmaceutical Chemistry, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for their warm encouragement.

It is my ardent duty to thank Honorable Chairperson Manamigu **Dr. K. Veeramani, M.A, B.L.,** Periyar College of Pharmaceutical Sciences, Tiruchirappalli for the completion of this work by means of providing the required facilities to my project.

I owe a warm hearted acknowledgement to our respected Correspondent **Thiru. Gnana. Sebastian,** Periyar College of Pharmaceutical Sciences, Tiruchirappalli for his encouragement towards my project work.

I am much indebted to **Mr. K. A. S. Mohammed Shafeeq, M.Pharm.,** Assistant Professor, Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for his valuable help during this research work.

I am also indebted to acknowledge and express my gratitude to **Dr. T. Shri Vijaya Kirubha, M.Pharm, Ph.D.,** Head, Department of Pharmacognosy Periyar College of Pharmaceutical Sciences, Tiruchirappalli for her kind co-operation in the Plant extraction and evaluation of Phytochemical screening

I express my gratitude to **Prof. G. Uma Devi, M.Sc, M.Phil.,** Head, Department of Medical Laboratory Technology, Periyar College of Pharmaceutical Sciences, Tiruchirappalli for her constructive support and guidance on antimicrobial screening work.

I express my earnest thanks to **Dr. P.Anantharaman, M.sc., M.Phill., M.Ed., Ph.D.** Associate Professor, **Dr. T. Ramesh,** Associate Professor. CAS in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai for their valuable help in authentication and collection of sample.

I extend my thanks to all the **Teaching and Non Teaching Faculty members** of Periyar College of Pharmaceutical Sciences, Tiruchirappalli for their valuable support.

Not as words but from the depth I thank my Parents for giving me unconditional support and motivation to pursue my interest even it went beyond the boundaries.

I would like to convey my forlorn mind for the innocent mice and rats without their immolation it was not possible to start my research work.

Finally I convey my thanks to everyone for their help in the completion of this research work successfully.

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**CENTRAL ANIMAL HOUSE REGISTRATION NUMBER: 265/2000/CPCSEA**

| | |
|---|---|
| Title of the project | : Pharmacological Evaluation of Marine Algae of Mandapam Coast :Ulva reticulata |
| Authors | : D.Kalivarathan & Dr. S. Karpagam Kumara Sundari |
| Proposal number | : PCP/IAEC/001/2017 |
| Date first received | : 03.11.2016 |
| Date received after modification (if any) | : 15.03.2017 |
| Date received after Second modification (if any) | : Nil |
| Approval date | : 15.05.2017 |
| Expiry date | : 15.05.2018 |
| Name of IAEC/ CPCSEA Chairperson | : The HoD Department of Pharmacology Periyar College of Pharmaceutical Sciences Trichy- 21 |

Date: 15. 05. 2017

CHAIRMAN

INSTITUTIONAL ANIMAL ETHICS COMMITTEE

PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES

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## ABBREVATIONS

| S. No. | Abbrevation | Expansion |
|---|---|---|
| 16. | ANOVA | Analysis of Variance |
| 17. | Ach | Acetyl choline |
| 18. | ATP | Adenosine tri Phosphate |
| 19. | approx. | Approximately |
| 20. | b.w | Body Weight |
| 21. | $Ca^{+}$ | Calcium ions |
| 22. | cAMP | Cyclic adenosine monophosphate |
| 23. | cGMP | Cyclic guanosine monophosphate |
| 24. | $Cl^{-}$ | Chloride ions |
| 25. | Cm | Centimeter |
| 26. | CPCSEA | Committee for the Purpose of Control and Supervision on Experimental Animals |
| 27. | DPPH | Diphenyl 2-picryl hydrazyl |
| 28. | EEG | ElectroEncephalograph |
| 29. | ETEC | Enterotoxigenic *Escherchia coli* |
| 30. | EPEC | Enteropathogenic *Escherchia coli* |
| 31. | Fig | Figure |
| 32. | GIT | Gastro Intestinal Tract |
| 33. | gm | Gram |
| 34. | $H^{+}$ | Proton |
| 35. | Hcl | Hydrochloric acid |
| 36. | hrs | Hours |
| 37. | HPTLC | High Performance Thin Layer Chromatography |
| 38. | H. pylori | Helicobacter pylori |
| 39. | i.v | Intravenous |
| 40. | $K^{+}$ | Potassium ions |
| 41. | kg | Kilogram |
| 42. | L | Litres |
| 43. | $LD_{50}$ | Lethal Dose |

| | | |
|---|---|---|
| 44. | ml | Millilitres |
| **45.** | **Abbrevation** | **Expansion** |
| 46. | mm | Milimeter |
| 47. | min | Minutes |
| 48. | mg | Milligram |
| 49. | mM | Milli Mole |
| 50. | µl | Microlitre |
| 51. | $Na^+$ | Sodium ions |
| 52. | Nacl | Sodium chloride |
| 53. | NSAIDs | Non Steroidal Anti Inflammatory Drugs |
| 54. | ORS | Oral Rehydration Salt |
| 55. | ORT | Oral Rehydration Therapy |
| 56. | PGs | Prostaglandins |
| 57. | PISE | Pilocarpine Induced Status Epilepticus |
| 58. | pH | Negative logarithm of hydrogen ion concentration |
| 59. | p.o. | per oral |
| 60. | ST | Stable toxin |
| 61. | SEM | Standard Error Mean |
| 62. | Sec | Seconds |
| 63. | TLC | Thin Layer Chromatography |
| 64. | T.s | Transverse section |
| 65. | TUM | Traditional Unani Medicine |
| 66. | wt | Weight |
| 67. | PTU | Propylthiouracil |
| 68. | $T_3$ | Thyroxine |
| 69. | $T_4$ | Triiodothyronine |
| 70. | TSH | Thyroid Stimulating Hormone |
| 71. | WHO | World Health Organisation |

# 1. INTRODUCTION

Pharmaceutical market is growing rapidly and continuously, but still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug resistant infectious diseases and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of ocean.[1]

The marine resources are nowadays widely studied because of numerous reasons. One of the reasons is as the ocean covers more than 70% of the world surface and among 36 known living phyla, 34 of them are found in marine environments with more than 300000 and known species of fauna and flora. The rationale of searching for drugs from marine environment stem from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous pressure including space competition, predation, surface fouling and reproduction.

The attention of finding drug from sea had started from 1970s. For instance, about 300 patents on bioactive marine natural products have been issued between 1969 and 1999. So far, more than 10,000 compounds have been isolated from marine organisms. Only 10% of over 25,000 plants have been investigated for biological activity. The marine environment may contain over 80% of world's plant and animal species

**Seaweeds [1]**

Seaweed is a loose colloquial term encompassing macroscopic, multicellular, benthic marine algae.

The term seaweed refers to the large marine algae that grow almost exclusively in the shallow waters at the edge of the world's oceans. They provide home and food for many different sea animals, lend beauty to the underwater landscape, and are directly valuable to man as a food and industrial raw material. Seaweeds are plants because they use the sun's energy to produce carbohydrates from carbon dioxide and water. They are simpler than the land plants mainly because they absorb the nutrients that they require from the surrounding water and have no need for roots or complex conducting tissues. Many types of seaweed have hollow, gas filled structures called floats or pneumatocysts. These help to keep the photosynthetic structures of the seaweed buoyant so they are able to absorb energy from the sun. The term thallus refers to the entire plant body of seaweed.

Seaweed draws an extraordinary wealth of mineral elements from the sea, which includes sodium, calcium, magnesium, potassium, chlorine, sulfur and phosphorus the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. It also contains several vitamins like carotenes (provitamin A), vitamin C, and vitamin $B_{12}$ along with higher proportion of essential fatty acids than land plants. Seaweeds provide a rich source of structurally diverse secondary metabolites, which includes terpenes, acetogenins, alkaloids and polyphenolics with many of these compounds being halogenated. The functions of these secondary metabolites are defense against herbivores, fouling organisms and pathogens they also play a role in reproduction, protection from UV radiation and as allopathic agents. Chemical defense mechanisms that inhibit bioflim development are a common occurrence in seaweeds, with many secondary metabolites produced by seaweeds having bacteriocidal or bacteriostatic properties. Physical stress such, as desiccation, UV and visible light and nutrient availability are able to alter the secondary metabolites in seaweeds

Seaweeds can be classified into three broad groups based on pigmentation

- Green seaweed (Chlorophyceae)
- Brown seaweed (Phacophyceae)
- Red seaweed (Rhodophyceae)

**Green seaweed (Chlorophyceae)**

The "green algae" is the most diverse group of algae, with more than 7000 species growing in a variety of habitats. The "green algae" is a paraphyletic group because it excludes the Plantae. Like the plants, the green algae contain two forms of chlorophyll, which they use to capture light energy to fuel the manufacture of sugars, but unlike plants they are primarily aquatic. Because they are aquatic and manufacture their own food, these organisms are called "algae," along with certain members of the Chromista, the Rhodophyta, and photosynthetic bacteria, even though they do not share a close relationship with any of these groups. Most green algae (which includes seaweeds) live in fresh water, so the saltwater-dwelling seaweeds make up a small portion of this group. Green seaweeds represent only about 10% of all green algae. Green seaweeds live mostly in the shallowest seawater, including intertidal pools that fill and drain with the tides. Some can live where salt water and fresh water mix, where rivers empty into the ocean. Green seaweeds often prefer warmer, tropical waters over coole.

A few other organisms rely on green algae to conduct photosynthesis for them. The chloroplasts in euglenids and chlorarachniophytes were acquired from ingested green algae, and in the latter retain a vestigial nucleus (nucleomorph). Green algae are also found symbiotically in the ciliate *Paramecium*, and in *Hydra viridis* and flatworms. Some species of green algae, particularly of genera *Trebouxia* and *Pseudotrebouxia* (Trebouxiophyceae) can be found in symbiotic associations with fungi to form lichens. In general the fungal species that partner in lichens cannot live on their own, while the algal species is often found living in nature without the fungus. *Trentepohlia* is a green alga living on humid soil, rocks or tree barks.

**Brown seaweed (Phacophyceae)**

Brown seaweed is alga that grows in cool oceans around the globe. You may have seen patches of its slimy green-brown branches washed up on the beach. Brown seaweed is a common ingredient in many Asian cuisines. People eat it raw, cooked, or pickled. If it's raw or pickled, it has a crisp crunch. Its texture is softer if it's cooked. Cooks serve up brown seaweed in a Korean soup called miyeok guk. You can also incorporate it into plain miso soup or seaweed salad. Its texture and flavor can make bland foods more interesting.

Worldwide there are about 1500–2000 species of brown algae. Some species are of sufficient commercial importance, such as *Ascophyllum nodosum* that they have become subjects of extensive research in their own right.

Brown algae belong to a very large group, the Heterokontophyta, a eukaryotic group of organisms distinguished most prominently by having chloroplasts surrounded by four membranes, suggesting an origin from a symbiotic relationship between a basal eukaryote and another eukaryotic organism. Most brown algae contain the pigment fucoxanthin, which is responsible for the distinctive greenish brown colour that gives them their name. Brown algae are unique among heterokonts in developing into multicellular forms with differentiated tissues, but they reproduce by means of flagellated spores andgametes that closely resemble cells of other heterokonts. Genetic studies show their closest relatives to be the yellow-green algae

**Red seaweed (Rhodophyceae)**

Red algae are red because of the presence of the pigment **phycoerythrin**; this pigment reflects red light and absorbs blue light. Because blue light penetrates water to a greater depth than light of longer wavelengths, these pigments allow red algae to photosynthesize and live at somewhat greater depths than most other "algae". Some

rhodophytes have very little phycoerythrin, and may appear green or bluish from the chlorophyll and other pigments present in them.

In Asia, rhodophytes are important sources of food, such as nori. The high vitamin and protein content of this food makes it attractive, as does the relative simplicity of cultivation, which began in Japan more than 300 years ago.

The red algae form a distinct group characterized by these attributes eukaryotic cells without flagella and centrioles, using floridean polysaccharides as food reserves with phycobiliproteins as accessory pigments (giving them their red color), and with chloroplasts lacking external endoplasmic reticulum and containing unstacked thylakoids. Most red algae are also multicellular, macroscopic, marine, and have sexual reproduction.

**Pharmacological properties of seaweeds [1]**

Many seaweeds and marine algae species have to contain significant ascorbic acid content. Apart from carbohydrates, fats and minerals, seaweeds are known to be rich in vitamins. Green seaweeds have been analyzed chemically for their fatty acid composition and altogether 36 fatty acids (18 saturated and 18 unsaturated fatty acids) were identified by gas chromatography and mass spectrometry.

The increased demand for medicines prepared from algae attracted the attention of scientist to utilize the algae as a source of medicine. The Chinese and Japanese have used seaweeds to treat goiter and other glandular problems since 300 B. C. The Roman used seaweeds to heal wounds, burns and rashes. The English used prophya to prevent scurvy on long voyages and chondrus for treatment of various internal disorders. Internal disorders such as constipation, stomach aches, and ulcers have been treated with chondrus, gracilaria and pteroclodra, all of these algae produce phytocolloids. The rhodophycean algae Digenia simplex is made into a drug in China as an antihelmenthic. The record of antibacterial product from algae was a substance chlorellin from chlorella. Extracts of vocca and walsh were effective against both gram positive and gram-negative bacteria. Seaweeds and other marine natural products contain variety of sterol, and sterols are known for their hypotensive activities. Himanthalia elongata a seaweed produce reduction of locomotor activity, hypermotility, pentylene-tetrazole induced convulsion on indicating significant CNS activity.

**Seaweed Products and Their Uses [1]**

Seaweeds are the only source for agar and algin and their use as food, fertilizer and fodder is well known in many parts of the world. Different products obtained from Indian seaweeds and their uses are given below.

**Agar**

This commercially important product is a colloidal carbohydrate present in the cell walls of certain red algae and it is a mixture of two polysaccharides, agarose and agaropectin. Humm and Yaphe have defined agar as a gel forming substance soluble in hot water and requiring one percent solution to set as a gel on cooling.

**Agaroids**

Gel-like extracts produced from certain types of red seaweeds are commonly known as agaroids. The carrageenans obtained from Chondrus and Gigartina species come under this group. Organic sulphate content is very much higher in these compounds and the chemical nature and properties of agaroids are different from agar agar. Pure solutions of agaroids are viscous and do not form gel when cooled as in the case of agar. However, various inorganic and organic solutes alter the properties and increase the gelling power of agaroids as observed in case of Hypnea extractive.

Carrageenan yielding plants seem to have not been reported from Indian waters, except for a rare and less abundant species of Gigartina (G. acicularis) occurring in the intertidal habits. But Hypnea musciformis, other spoecies of Hypnea, Spyridia, Sarconema, Acanthophora, Laurencia and Chondria growing along the Indian coast give gel-like extracts and some preliminary studies have been made at the Central Marine Fisheries Research Institute on these plants [2] (Pillai, 1957b; Thivy, 1951). From the information available it is evident that the yield of Sarconema filiforme extractive is 10% with a gel strength of 5gm/cm 2 and a gelation temperature of 380 C for 1.5% solution[3] (Thivy, 1951). In the extractive of Hypnea Musciformis (Rama Rao and Krishnamurthy, 1968) gel formation was not seen in 1.0% solution[4].

**Algin**

Algin or alginic acid is the main polysaccharide occurring in the cell walls of brown algae. It consists of D-manmuronic acid and 2- guluronic acid in various proportions. The sodium, potassium and magnesium salts of alginic acid are soluble in water and they give viscous solutions without gel formation. Calcium alginate and other salts of copper, cobalt, mercury etc. are insoluble in water.

Species of Sargassum, Turbinaria, Cystophyllum, Hormophysa, Dictyota and Padina Are some of the brown weeds reported from the Indian waters. Of these, Sargassum is the principal source for the production of algin in the country. In the laboratory tests conducted at the Central Marine Fisheries Research Institute Turbinaria was also found to be a good source for algin preparation, but its occurrence is restricted to certain areas of the coastline. The yield of alginic acid varied from species to species and Sargassum tenerrimum, Sargassum wightii, Sargassum swartzii, Sargassum cinereum, Sargassum johnstonii, Turbinaria conoides and Turbinaria ornate are some of the high yielding varieties occurring along the Indian coast.

**Uses of Agar and Algin**

In general both agar and algin serve as stabilizers, emulsifiers, thickeners gelling agents. Agar is often used where firm gel is needed and algin for soft and viscous products. In ice cream industry both agar and algin are used as stabilizing agents to give smooth body and texture to the ice creams and also to prevent the formation of large ice crystals. Similarly these two seaweed colloids are employed in icings to prevent adhesion of the sugar coating to wrappers, in canning industry as coating materials for preserving fish, meat and other products, in the preparation of milk puddings, sherbats, dental impression materials and agricultural sprays. Some information on the food products requiring agar and their preparation.

**Algal proteins**

Some green and red seaweeds such as *Ulva fasciata, Ulva rigida, Porphyra vietnamensis, Acanthophora muscoides* and *Centroceras clavulatum* are very rich in proteins. These algal proteins have many essential amino acids, including iodine containing amino acids. Studies have shown that the weeds mentioned above contain 16-30% of protein on dry weight basis and this amount is somewhat higher than in other food materials like cereals and eggs. Protein concentrates can be prepared by extracting protein from these green and red seaweeds, and dry powders of *Ulva, Porphyra, Acanthophora* etc. can be added to various foods of protein deficiency or consumed in small quantities along with the other food stuffs.

**Seaweed meal**

As seaweeds are cheap sources for minerals and trace elements, meals prepared from seaweeds can be utilized as supplements in daily foods for cattle, poultry and other farm animals. Seaweed meals can be prepared by pulverizing the cleaned and washed

weeds. Thivy has described a simple method for the preparation of seaweed meal from *Gracilaria lichenoides.*

**Seaweed manure**

From time immemorial seaweeds have been used as manure in the coastal areas. Important feature of seaweed manure is that the minerals and trace elements occur in water soluble form and when the manure is applied the plants readily absorb these chemical constituents. Carbohydrates and other organic matter occurring in seaweeds alter the nature of the soil and increase its moisture holding capacity. The minerals and trace elements present in the seaweed manure also control deficiency diseases in plants.

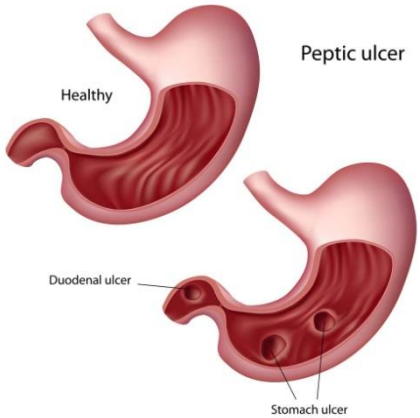
Large quantities of seaweeds and sea grasses like *Cymadocea, Diplanthera* and *Halophila* can be utilized as manure directly or in the form of compost. A method for composting the seaweeds with cow dung has been described by Thivy. Field experiments have been conducted in the Central Marine Fisheries Research Institute applying seaweed compost to bhendi, sweet potato, tapioca, brinjal plants and high yields were obtained from these vegetables crops. In certain coastal areas of Tamilnadu, cast ashore weeds are used as manure for coconut plantations.

## Disease Profile

**Stomach Ulcer**[5]

Stomach ulcers are painful sores that can be found in the stomach lining or small intestine. Stomach ulcers are the most visible sign of peptic ulcer disease. They occur when the thick layer of mucus that protects your stomach from digestive juices is reduced, thus enabling the digestive acids to eat away at the lining tissues of the stomach.

**Fig. No. 1: Stomach Ulcer**



**Pathophysiology[6]**

Peptic ulcers result from an imbalance between factors that can damage the gastroduodenal mucosal lining and defense mechanisms that normally limit the injury. Aggressive factors include gastric juice (including hydrochloric acid, pepsin, and bile salts refluxed from the duodenum), *H pylori*, and NSAIDs. Mucosal defenses comprise a mucus bicarbonate layer secreted by surface mucus cells forming a viscous gel over the gastric mucosa; the integrity of tight junctions between adjacent epithelial cells; and the process of restitution, whereby any break in the epithelial lining is rapidly filled by adjacent epithelial and mucosal stromal cells migrating and flattening to fill the gap. Mucosal defenses depend on an adequate blood supply and on formation within the gastric mucosa.

In general, duodenal ulcers are the result of hypersecretion of gastric acid related to *H pylori* infection (the majority of cases), whereas secretion is normal or low in patients with gastric ulcers.

In duodenal ulcers, chronic *H pylori* infection confined mainly to the gastric antrum leads to impaired secretion of somatostatin and consequently increased gastrin release, resulting in gastric acid hypersecretion. In Zollinger-Ellison syndrome, a gastrin-secreting neuro-endocrine tumour is the stimulus for high rates of gastric acid secretion.

In gastric ulcers, longstanding *H pylori* infection throughout the stomach accompanied by severe inflammation results in gastric mucin degradation, disruption of tight junctions between gastric epithelial cells, and the induction of gastric epithelial cell death. NSAIDs cause injury directly (involving trapping hydrogen ions) and indirectly (a systemic effect involving the inhibition of cyclo-oxygenases, especially COX-1) and increase bleeding risk through anti platelet actions. Chronic gastric ischaemia underlies the stress ulcers of patients in intensive care.

**Types of Ulcer[6]**

- Peptic Ulcer
- Aphthous Ulcers
- Esophageal Ulcers

**Peptic Ulcer**

Peptic ulcer is a broad term which includes ulcers of digestive tract in the stomach or the duodenum. Earlier it was believed that one developed this type of ulcers due to stress and spicy food. However, recent research has shown that these are just the

aggravating factors. The causative agent is infection caused by the bacteria H. pylori or reaction to certain medicines like non steroidal anti -inflammatory drugs (NSAIDs) Symptoms of peptic ulcers include abdominal discomfort and pain. Other symptoms include weight loss, poor appetite, bloating, nausea, and vomiting. Some may also experience blood in stool and vomit, and black stools that indicate gastrointestinal bleeding.

**Aphthous Ulcers**

Sores that develop in the inner lining of the mouth are referred to as mouth ulcers. Mouth ulcers are common and are usually due to trauma such as from ill fitting dentures, fractured teeth, or fillings. Anemia, measles, viral infection, oral candidiasis, chronic infections, throat cancer, mouth cancer and vitamin B deficiency are some of the common causes of ulcers or sores in the mouth. Aphthous minor is amongst the most common form of oral ulcerative diseases and affects an estimated 15-20% of the population worldwide. In some populations, the prevalence has been documented as being as high as 50-66% and it is especially common in North America The incidence of aphthous ulcers has been found to be lower in smokers than in non-smokers.

**Esophageal Ulcers**

Esophageal ulcers are lesions that occur in the esophagus (the food pipe). These are most commonly formed at the end of the food pipe and can be felt as a pain right below the breastbone, in the same area where symptoms of heartburn are felt. Esophageal ulcers are associated with acid reflux or GERD, prolonged use of drugs like NSAIDs, and smoking**.**

**Causes of stomach ulcers**[7]

Stomach ulcers aren't necessarily caused by one single factor. The decrease in the stomach's mucus lining that leads to an ulcer is usually caused by one of the following :

- an infection with the bacterium *Helicobacter pylori* (*H. pylori*)
- long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen
- excess acid (hyperacidity) in the stomach, which may be related to genetics, lifestyle (stress, smoking), and certain foods
- Zollinger-Ellison syndrome, a rare disease that makes the body produce excess stomach acid

**Certain factors and behaviors can put you at higher risk for developing stomach ulcers[8]**

- smoking
- frequent use of steroids (such as those for treating asthma)
- hypercalcemia (overproduction of calcium)
- family history of stomach ulcers
- excessive consumption of alcohol

**Helicobacter pylori**

H. pylori is the etiologic factor in most patients with peptic ulcer disease and may predispose individuals to the development of gastric carcinoma. H. pylori colonizes in the human stomach.The method of H. pylori transmission is unclear, but seems to be person-to-person spread via a fecal-oral route. The prevalence of H. pylori in adults appears to be inversely related to the socioeconomic status. It is also thought that water is a reservoir for transmission of H. pylori.

**Hypercalcemia**

Hypercalcemia has a direct bearing on the gastric acid hypersecretory state found in patients with Zol linger-Ellison syndrome and MEN I. Intravenous calcium infusion in normal volunteers induces gastric acid hypersecretion. Additionally, calcium has been demonstrated in vivo and in vitro to stimulate gastrin release directly from gastrinomas. Resolution of hypercalcemia (by parathyroidectomy) reduces the basal acid output and serum gastrin concentration in fasting gastrinoma patients and those with MEN I, suggesting that resolution of hypercalcemia plays an important role in the therapy of this subgroup of patients.

**Genetic factors**

Genetic factors play a role in the pathogenesis of ulcer disease. The lifetime prevalence of developing ulcer disease in first-degree relatives of ulcer patients is about three times greater than the general population. Approximately 20–50% of duodenal ulcer patients report a positive family history; gastric ulcer patients also report clusters of family members who are likewise affected.

**Smoking**

The literature reveals a strong positive correlation between cigarette smoking and the incidence of ulcer disease, mortality, complications, recurrences and delay in healing rates. Smokers are about two times more likely to develop ulcer disease than nonsmokers. Cigarette smoking and H. pylori are co-factors for the formation of peptic ulcer disease.

There is a strong association between H. pylori infection and cigarette smoking in patients with and without peptic ulcers. Cigarette smoking mayincrease susceptibility, diminish the gastric mucosal defensive factors, or may provide a more favorable milieu for H. pylori infection.

**Stress**

Numerous studies have revealed conflicting conclusions regarding the role of psychological factors in the pathogenesis and natural history of peptic ulcer disease.The role of psychological factors is far from established. Acute stress results in increases in pulse rate, blood pressure and anxiety, but only in those patients with duodenal ulcers did acute stress actually result in significant increases in basal acid secretion. There is no clearly established "ulcer-type" personality. Ulcer patients typically exhibit the same psychological makeup as the general population, but they appear to perceive greater degrees of stress. In addition, there is no evidencethat distinct occupational factors influence the incidence of ulcer disease.

**Alcohol and Diet**

Although alcohol has been shown to induce damage tothe gastric mucosa in animals, it seems to be related to the absolute ethanol administered (200 proof). Pure ethanol is lipid soluble and results in frank, acute mucosal damage. Because most humans do not drink absolute ethanol, it is unlikely there is mucosal injury atethanol concentrations of less than 10% (20 proof).Ethanol at low concentrations (5%) may modestly stimulate gastric acid secretions; higher concentrations diminish acid secretion. Though physiologically interesting,this has no direct link to ulcerogenesis or therapy.Some types of food and beverages are reported to cause dyspepsia. There is no convincing evidence that indicates any specific diet causes ulcer disease.Epidemiologic studies have failed to reveal a correlation between caffeinated, decaffeinated, or cola-type beverages, beer, or milk with an increased risk of ulcer disease. Dietary alteration, other than avoidance of pain-causing foods, is unnecessary in ulcer patients.

**Symptoms of stomach ulcers**

A number of symptoms are associated with stomach ulcers. The severity of the symptoms depends on the severity of the ulcer. The most common symptom is a burning sensation or pain in the area between your chest and belly button. Normally, the pain will be more intense when your stomach is empty and it can last for a few minutes or several hours

**Other common symptoms include**:

- dull pain in the stomach
- weight loss
- not wanting to eat because of pain
- nausea or vomiting
- bloating
- burping or acid reflux
- heartburn (burning sensation in the chest)
- pain improves when you eat, drink, or take antacids

**Diagnosis**[9]

All peptic ulcers aren't caused by bacteria, it's getting more common to do a test for *Helicobacter pylori* test for *Helicobacter pylori* whenever someone has ulcer symptoms. This includes testing blood, breath, stool, or a sample of tissue from your digestive tract (biopsy)

- Endoscopy
- Biopsy

If a person is older than 55 yrs, may need an endoscopy because of a higher risk for stomach cancer. This is especially true if the person have

- Ulcer symptoms for the first time.
- Ulcer symptoms that return before or after treatment is completed.
- A family history of stomach cancer.

Other symptoms that may point to a more serious problem, such as stomach cancer. These include

- Blood in the stool.
- Weight loss of more than 10% of body weight.
- Anemia.
- Difficulty swallowing (dysphagia).
- Jaundice.
- Abdominal mass.

Other tests that may be done include

- Fecal Occult Blood Test (FOBT)
- Complete Blood Count (CBC)
- Upper GI series

**Peptic Ulcer Disease-Therapy[10,11]**

- Medicaltherapy
- Surgery
- EndoscopicTherapy

**Medical therapy**

**1) Antacids -**Largedosesrequired 1and3 hours after meals, magnesiumhydroxide-diarrhoea

**2) Histamine H2-receptor antagonists -**cimetdine, ranitidine, famotidine and nizatidine

**3) Protonpumpinhibitors-**Resistant to other therapies,preventNSAID-gastroduodenalulcers,omeprazolelansoprazole

**4) Prostaglabdinstimulators-** Sucralfate,Misoprostol

**5) Anti - *H. pylori* drugs:** Amoxicillin, Clarithromycin, Metronidazole, Tinidazole, Tetracycline

**$H_2$ Receptor Antagonists**

These are the first class of highly effective drugs for acid-peptic disease, but have been surpassed by proton pump inhibitors (PPIs). Four $H_2$ antagonists cimetidine, ranitidine, famotidine and roxatidine are available in India; many others are marketed elsewhere. Their interaction with $H_2$ receptors has been found to be competitive in case of cimetidine, ranitidine and roxatidine, but competitive-noncompetitive in case of famotidine. Cimetidine was the first $H_2$ blocker to be introduced clinically and is described as the prototype, though other $H_2$ blockers are more commonly used now.

**Uses**

The $H_2$ blockers are used in conditions in which it is profitable to suppress gastric acid secretion. Used in appropriate doses, all available agents have similar efficacy. However, PPIs, because of higher efficacy and equally good tolerability, have outstripped $H_2$ blockers.

**Duodenal ulcer**

$H_2$ blockers produce rapid and marked pain relief (within 2- 3 days) 60- 85% ulcers heal at 4 weeks and 70- 95% ulcers at 8 weeks, but they are seldom used now to heal existing ulcers. Suppression of nocturnal secretion by single high bed time dose is equally efficacious and physiologically more sound. About ½ of the patients relapse within 1 year of healing with $H_2$ blockers. Maintenance therapy with bed time dose reduces the relapse rate to 15- 20% per year as long as given.

**Proton Pump Inhibitors (PPIs)**

**Omeprazole**

It is the prototype member of substituted benzimidazoles which inhibit the final common step in gastric acid secretion. The PPIs have overtaken $H_2$ blockers for acid-peptic disorders. The only significant pharmacological action of omeprazole is dose dependent suppression of gastric acid secretion, without anticholinergic or $H_2$ blocking action. It is a powerful inhibitor of gastric acid: can totally abolish HCl secretion, both resting as well as that stimulated by food or any of the secretagogues, without much effect on pepsin, intrinsic factor, juice volume and gastric motility. Omeprazole is inactive at neutral pH, but at pH < 5 it rearranges to two charged cationic forms (a sulphenic acid and a sulphenamide configurations) that react covalently with SH groups of the $H^+K^+$ATPase enzyme and inactivate it irreversibly, especially when two molecules of omeprazole react with one molecule of the enzyme. After absorption into bloodstream and subsequent diffusion into the parietal cell, it gets concentrated in the acidic pH of the canaliculi because the charged forms generated there are unable to diffuse back. Moreover, it gets tightly bound to the enzyme by covalent bonds. These features and the specific localization of $H^+K^+$ATPase to the apical membrane of the parietal cells confer high degree of selectivity of action to omeprazole. Acid secretion resumes only when new $H^+K^+$ATPase molecules are synthesized (reactivation half time 18 hours). It also inhibits gastric mucosal carbonic anhydrase.

**Uses**

**Peptic ulcer**

Omeprazole 20 mg OD is equally or more effective than $H_2$ blockers. Relief of pain is rapid and excellent. Faster healing has been demonstrated with 40 mg/day: some duodenal ulcers heal even at 2 weeks and the remaining (over 90%) at 4 weeks. Gastric

Ulcer generally requires 4- 8 weeks. It has caused healing of ulcers in patients not responding to $H_2$ blockers. Continued treatment (20 mg daily or thrice weekly) can prevent

ulcer relapse. PPIs are an integral component of anti-*H. pylori* therapy. Proton Pump Inhibitors (PPIs) are the drugs of choice for NSAID induced gastric/duodenal ulcers. Healing may occur despite continued use of the NSAID. However, higher doses given for longer periods are generally required. When the NSAID cannot be stopped, it is advisable to switch over to a COX-2 selective NSAID. Maintenance PPI treatment reduces recurrence of NSAID associated ulcer.

**Bleeding peptic ulcer**

Acid enhances clot dissolution promoting ulcer bleed. Suppression of gastric acid has been found to facilitate clot formation reducing blood loss and rebleed. High dose i.v. PPI therapy (Pantoprazole 40 -120 mg/day or Rabeprazole 40- 80 mg/day) profoundly inhibits gastric acid, and has been shown to reduce rebleeding after therapeutic endoscopy. Even in cases where the bleeding vessel could not be visualized, i.v. followed by oral PPI reduces recurrence of bleeding and need for surgery.

**Stress ulcers**

Intravenous Pantoprazole/ Rabeprazole is as effective prophylactic (if not more) for stress ulcers as *i.v.* $H_2$ blockers.

**Gastroesophageal reflux disease (GERD)**

Omeprazole produces more complete round-the-clock inhibition of gastric acid resulting in rapid symptom relief and is more effective than $H_2$ blockers in promoting healing of esophageal lesions. PPIs are the drugs of choice. Higher doses than for peptic ulcer or twice daily administration are generally needed.

**Zollinger-Ellison syndrome**

Omeprazole is more effective than $H_2$ blockers in controlling hyperacidity in Z-E syndrome. However, 60-120 mg/day or more (in 2 divided doses) is often required for healing of ulcers. Inoperable cases have been treated for >6 years with sustained benefit and no adverse effects. Other gastric hypersecretory states like systemic mastocytosis, endocrine adenomas, etc. also respond well.

**Aspiration pneumonia**

PPIs are an alternative to $H_2$blockers for prophylaxis of aspiration pneumonia due to prolonged anaesthesia. Omizac, Nilsec 20 Mg Cap. Omez, Ocid, Omezol 10,20 mg caps, Protoloc 20,40 mg caps containing enteric coated granules. Capsules must not be opened or chewed; to be taken in the morning before meals.

**Adverse effects**

PPIs produce minimal adverse effects. Nausea, loose stools, headache, abdominal pain, muscle and joint pain, dizziness are complained by 3- 5%. Rashes (1.5% incidence), leucopenia and hepatic dysfunction are infrequent. On prolonged treatment atrophic gastritis has been reported occasionally. No harmful effects of PPIs during pregnancy are known. Though manufacturers advise to avoid, PPIs have often been used for Gastro Esophageal Reflux Disease (GERD) during pregnancy.

**Lansoprazole**

Lansoprazole is Somewhat more potent than omeprazole but similar in properties. Inhibition of $H^+$ $K^+$ATPase by lansoprazole is partly reversible. It has higher oral bioavailability, faster onset of action and slightly longer t½ than omeprazole. Dose should be reduced in liver disease. Side effects are similar, but drug interactions appear to be less significant; diazepam and phenytoin metabolism may be reduced. Ulcer healing dose*:* 15-30 mg OD; Lanzol, Lanzap, Levant, Lanpro 15, 30 mg caps\ Prostaglandin Analoguepge2 and $PGI_2$ are produced in the gastric mucosa and appear to serve a protective role by inhibiting acid secretion and promoting mucus as well as $HCO_3^-$ secretion. In addition, PGs inhibit gastrin release, increase mucosal blood flow and probably have an illdefined "cytoprotective" action. However, the most important appears to be their ability to reinforce the mucus layer covering gastric and duodenal mucosa which is buffered by $HCO_3^-$ secreted into this layer by the underlying epithelial cells.

**Drugs *Helicobacter pylori* Infection**

*H. pylori* is a gram negative bacillus uniquely adapted to survival in the hostile environment of stomach. It attaches to the surface epithelium beneath the mucus, has high urease activity produces ammonia which maintains a neutral microenvironment around the bacteria, and promotes back diffusion of $H^+$ ions. It has been found as a commensal in 20-70% normal individuals, and is now accepted as an important contributor to the causation of chronic gastritis, dyspepsia, peptic ulcer, gastric lymphoma and gastric carcinoma. *H. pylori* infection starts with a neutrophilic gastritis lasting 7- 10 days which is usually asymptomatic. Once established, *H. pylori* generally persist for the life of the host. Up to 90% patients of duodenal and gastric ulcer have tested positive for *H. pylori*.

Eradication of *H. pylori* concurrently with $H_2$ blocker/PPI therapy of peptic ulcer has been associated with faster ulcer healing and largely prevents ulcer relapse. All *H. pylori* positive ulcer patients should receive *H. pylori* eradication therapy. In the absence

of *H. pylori* testing, all cases with failed conventional ulcer therapy and relapse cases must be given the benefit of *H. pylori* eradication.

Antimicrobials that are used clinically against *H. pylori* are Amoxicillin, clarithromycin, Tetracycline and Metronidazole/ Tinidazole. However, any single antibiotic is ineffective.Resistance develops rapidly, especially to metronidazole/tinidazole and clarithromycin, but amoxicillin resistance is infrequent. In tropical countries, Metronidazole resistance is more common than Clarithromycin resistance. Since bismuth (CBS) is active against *H. pylori* and resistance does not develop to it, combination regimens including bismuth may be used in case of Metronidazole and Clarithromycin double resistance. Routine use of CBS is precluded by poor patient acceptability. Acid suppression by PPIs/$H_2$ blockers enhances effectiveness of anti-*H. pylori* antibiotics, and optimum benefits are obtained when gastric pH is kept > 5 for at least 16 - 18 hours per day. This is a higher degree of round-the clock acid suppression than is needed for duodenal ulcer healing or for reflux esophagitis. Only twice daily PPI dosing can achieve this degree of acid suppression. The PPIs benefit by altering the acid environment for *H. pylori* as well as by direct inhibitory effect. One of the PPIs is an integral component of all anti-*H. pylori* regimens along with 2 (triple drug) or three (quadruple drug) antimicrobials. A number of 3 drug regimens of 1 or 2 weeks are being used. One week regimens are adequate for many patients, but 2 week regimens achieve higher (upto 96%) eradication rates, though compliance is often poor due to side effects.

**Anti - *H. pylori* Regimens**

Proton pump inhibitor Amoxicillin Clarithromycin Metronidazole/Tinidazole The US-FDA approved regimen is: Lansoprazole 30 mg + Amoxicillin 1000 mg + clarithromycin 500 mg, all given twice daily for 2 weeks. This has achieved 86–92% eradication rate. Better tolerability of regimens which exclude a nitroimidazole favour using amoxicillin + clarithromycin + PPI, particularly in India where metronidazole resistance is more prevalent. However, for the sake of simplicity and economy, the National Formulary of India (NFI, 2010) suggests a model *H. pylori* eradication regimen of 1 week consisting of: Omeprazole 40 mg OD + Metronidazole 400 mg TDS + Amoxicillin 500 mg TDS. For large ulcers (> 10 mm in diameter) or those complicated by bleeding/perforation, the PPI should be continued beyond the 2 weeks-triple drug regimen till complete healing occurs. For patients who have, in the near past, received a nitroimidazole (for other infections) or a macrolide antibiotic, metronidazole or clarithromycin, as the case may be, should be excluded. Quadruple therapy with CBS 120

mg QID + Tetracycline 500 mg QID + Metronidazole 400 mg TDS + Omeprazole 20 mg BD is advocated for eradication failure cases. All regimens are complex and expensive, side effects are frequent and compliance is poor. Higher failure rates (20- 40%) of *H. pylori* eradication have been reported from India. Also, 5 year recurrence rate of *H. pylori* infection is higher. Three week treatment is being advocated by some. Nevertheless, long - term benefits of anti - *H. pylori* therapy include lowering of ulcer disease prevalence and prevention of gastric carcinoma/lymphoma, but benefits in nonulcer dyspepsia are equivocal. *H. pylori* vaccines are under development.

**Antacids**

Antacids are often prescribed to help neutralize stomach acid. It is important that individuals do not try to treat their ulcer (or suspected ulcer) without medical advice, because some of the over-the-counter medications may cause more gastric acid production.Also, such medications may mask the symptoms of underlying serious disease.

Prescription drugs may be used to either stimulate better mucus production, coat the stomach with other protective substances, neutralize acid, or actually slow down the production of acid.It is important not to take medication prescribed for someone else, because serious side effects may occur.

**AntibioticTherapy** has become a new option for medical treatment of ulcers. The exciting discovery of the role of *Helicobacter pylori* bacterium held the promise that antibiotic treatment would be a major treatment breakthrough for those who have ulcers .Early research studies indicated that antibiotics could eliminate the bacterium, thereby preventing recurrences of ulcers.Antibiotic treatment is not automatically prescribed for every ulcer patient, however, because many ulcer patients do not have the bacteria.

**Endoscopy**

Endoscopy may be used to treat ulcers in those severe cases where the ulcer has penetrated through the lining of the GI tractto deeper tissue.When an ulcer has developed to that extent, bleeding may occur.An endoscope can be passed into the GItract from the mouth and can be used to stop (coagulate) bleeding vessels.Often this procedure can help avoid surgery

**Diet**

In the past, an ulcer diet meant a strict bland diet with lots of milk and dairy products .That type of diet was thought to help an ulcer heal. Research has shown that some foods that at first seem to neutralize stomach acid actually stimulate production of

more acid, so even though drinking milk may cause quick relief, a few hours later, the problem may grow worse than before.Now that we know that dairy products increase stomach acid production, milk-based diets are no longer used for treatment.

**Injection Therapy**

Injection therapy for upper gastrointestinal bleeding is inexpensive, simple and widely used. A sclerotherapy catheter with a small retractable needle is passed through the biopsy channel of the endoscope. Non-bleeding visible vessels are treated by the injection of a solution at three or four surrounding sites about 1 - 3 mm from the vessel. Subsequently, the visible vessel is injected. In cases of bleeding vessels, injections are made around the bleeding point until hemostasis is achieved. This is followed by injection into the vessel

Several different sclerosant agents have been used alone or in combination to achieve endoscopic hemostasis. Adrenaline; hypertonic saline and adrenaline combined; adrenaline and polidocanol; pure ethanol; or combinations of dextrose, thrombin, and sodium morrhuate have shown improvement in rebleeding, the need for urgent surgery, and mortality.

Combined injection and thermal treatment have theoretical advantages in the treatment of bleeding ulcers. Injection with epinephrine produces vasoconstriction and activates platelet coagulation, reducing blood flow and potentiating thermal therapy, which produces coaptive coagulation. Recent studies have shown combination therapy (epinephrine injection and heater probe) benefited patients with spurting bleeding, but not those with oozing bleeding

**Mechanical Therapy**

Endoscopic hemoclips have recently been developed and made their way to the scene of endoscopic therapy for peptic ulcer disease. These devices are small 3-4mm titanium clips that can be opened and closed while being operated through the working channel of the endoscope. They may be used to pinch off, or clip, ableeding vessel. When fully deployed, they remain fastened to the vessel after the endoscope has been removed from the patient. Emerging studies have shown that hemoclips are an effective and safe method for treating certain forms of peptic ulcer desease and should be used in the appropriate setting.

**Radiological Therapy**

Angiography is a useful diagnostic and therapeutic modality in treatment of bleeding gastric and duodenal ulcers. Angiography can identify the site of bleeding in

instances where endoscopy has failed to be diagnostic. It should also be considered in patients at high risk for surgical intervention.Angiographic therapy includes two different embolization techniques for the treatment of GI bleeding. Effective in 50% of cases, vasopressin intra-arterial infusion causes vasoconstriction that results in the cessation of ulcer hemorrhage. Embolic material such as an absorbable gelatin sponge, tissue adhesives, or other occlusion devices (such as microcoils) can be inserted through a catheter into the area of bleeding.

**Surgical Therapy**

When endoscopic hemostasis techniques are unavailable or fail to resolve bleeding or recurrent hemorrhage, surgery provides another therapeutic option. Surgery is effective in the prevention of recurrent ulceration and in excluding the presence of malignant disease. Emergent surgery has a higher mortality rate than elective surgery, and resection procedures are accompanied by higher mortality than oversewing the ulcer and selective vagotomy, or vagotomy and pyloroplasty. The operative choice is related to the surgeon's experience, ulcer location, and overall condition of the patient. Truncal vagotomy and antrectomy provide high cure rates and low recurrence rates. Recurrence rates after vagotomy and pyloroplasty are somewhat higher. Laparoscopic selective vagoto my provides an appealing alternative for a subset of ulcer patients with lower morbidity, shorter recovery time, and a shorter hospital stay.

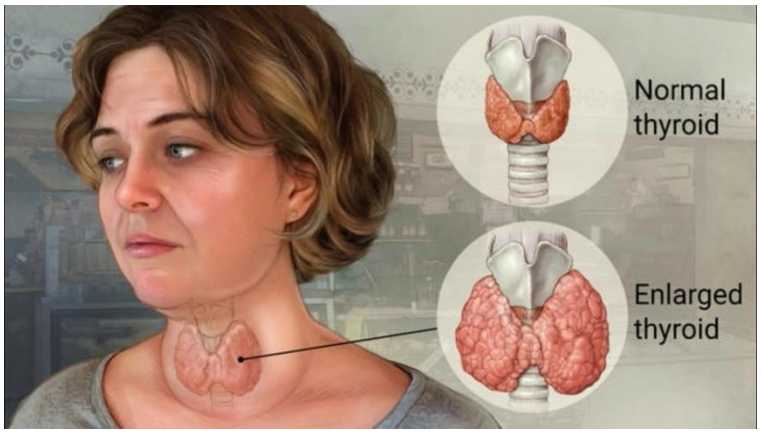
**Goiter[12]**

Goiter is the enlargement of the thyroid gland and is not cancerous. Simple endemic goiter is usually caused by lack of iodine in the diet. A person with goiter can have normal levels of thyroid hormone (euthyroidism), excessive levels (hyperthyroidism) or levels that are too low (hypothyroidism)

The thyroid is a gland, shaped like a butterfly, located at the base of the neck, just below the Adam's apple. If the thyroid gland grows larger than normal the patient has a condition known as goiter. The thyroid gland produces a hormone called thyroxine, under the influence of another hormone called TSH, which is produced by the pituitary gland in the brain. Thyroxine ($T_4$) and Triiodothyronine ($T_3$) which is formed from thyroxine are responsible for goiter.

Goiters are generally painless however if the gland gets very large the patient may have problems swallowing properly and may also develop a cough. Goiter refers both to the enlarged thyroid and the condition of having an enlarged thyroid gland.

**Fig. No. 2: Normal and goiter affected thyroid gland**




Goiter is a chronic enlargement of the thyroid gland, not due to a neoplasm, occurring endemically in certain localities, especially regions where glaciation occurred and the soil is low in iodine, and sporadically elsewhere

**Pathophysiology[13]**

The thyroid gland is controlled by thyroid-stimulating hormone (TSH; also known as thyrotropin), secreted from the pituitary gland, which in turn is influenced by the thyrotropin-releasing hormone (TRH) from the hypothalamus. TSH permits growth, cellular differentiation, and thyroid hormone production and secretion by the thyroid gland. Thyrotropin acts on TSH receptors located on the thyroid gland. Thyroid hormones are synthesized from iodination of tyrosine. The iodine is transported from plasma into the thyroid cell via a sodium-iodide symporter. This is an active process resulting in an intracellular iodine level exceeding 20 times the plasma iodine level. This iodine transport activity is controlled by TSH. Serum thyroid hormones levothyroxine and triiodothyronine feed back to the pituitary, regulating TSH production. Interference with this TRH-TSH thyroid hormone axis causes changes in the function and structure of the thyroid gland. Stimulation of the TSH receptors of the thyroid by TSH, TSH-receptor antibodies, or TSH receptor agonists, such as chorionic gonadotropin, may result in a diffuse goiter. When a small group of thyroid cells, inflammatory cells, or malignant cells metastatic to the thyroid is involved, a thyroid nodule may develop.

A deficiency in thyroid hormone synthesis or intake leads to increased TSH production. Increased TSH causes increased cellularity and hyperplasia of the thyroid gland in an attempt to normalize thyroid hormone levels. If this process is sustained, a goiter is established. Causes of thyroid hormone deficiency include inborn errors of thyroid hormone synthesis, iodine deficiency, and goitrogens.

A goiter may result from a number of TSH receptor agonists. TSH receptor stimulators include TSH receptor antibodies, pituitary resistance to thyroid hormone, adenomas of the hypothalamus or pituitary gland, and tumors producing human chorionic gonadotropin.

**Types of Goitre[14]**

- In diffuse goitre the whole thyroid gland swells and its smooth to touch
- In nodular goitre, solid or fluid-filled lumps called thyroid nodules develop in the thyroid gland.

**Nodular goitre is further classified into two types**

- **Uninodular** : with only one nodule
- **Multinodular** : with more than one nodule
- **Endemic goiter**: occurs due to insufficient dietary iodine intake. More than 10% of the community is usually affected.
- **sporadic goitre:** a lesser number of individuals from the community are affected. The risk factors include a positive family history, dietary iodine deficiency, age (over 40 years) and female gender.

**Iodine Deficiency Disorders[15]**

**Iodine**

Iodine is an essential trace element. It present in the body in a minute amount (normally 20-30mg). 80% of the iodine in the body (15mg in adult) is present in the thyroid gland where it is used in the synthesis of several thyroid hormones. The remainder is distributed throughout other tissues, particularly in the lactating mammary, salivary, gastric glands and in the kidneys. Within the circulation iodine occurs in the form of free iodine ion or as protein bound iodine. Excretion is primarily in urine and small amount in feces.

**Sources**

Iodine is provided in the diet by food and water. The iodine in the water occurs in form of iodide ion in amount that varies from region to region in line with variation in iodine content of the soil. These variations in iodine content of the soil are also reflected in the variations in the iodine content of the plant and animals raised on the soil. Sea food and salt water fish are rich sources (30 - 300µg/100gm meat) next come fresh water fish (20 - 40µg/ 100 gm meat).

**Recommended intake**

| | |
|---|---|
| Adult (male &female) | : 150µg/day |
| Pregnant women | : 175µg /day |
| Lactating women | : 200µg /day |
| Children | : 40µg /day |

**Function of iodine**

- It is an integral part of thyroid hormones that play a major role in regulating growth and development. They also have important role in regulation of metabolic rate
- It is required for early development of nervous system during fetal life
- It is needed for normal reproductive function

**Iodine deficiency**

It is the leading cause of preventable intellectual impairment. It is associated with a variety of clinical disorders called "iodine deficiency disorders". Iodine deficiency disorder is known to be significant health problem in 118 countries, iodine deficiency disorder affects 740 million of people (13% of world population) and 30% of remainder is at risk.

Iodine deficiency disorder affect poor pregnant women, preschool children causing serious health problems that includes endemic goiter, endemic cretinism, hypothyroidism, mental retardation, reproductive failure, abortion and still birth, childhood mortality and socio-economic retardation.

**Endemic goiter**

- Enlargement of thyroid gland, most obvious clinical manifestation of iodine deficiency caused by dietary deficiency of iodine
- The minimum amount of iodine required to cover the turnover of the thyroid gland is 50µg/ day
- Below this the thyroid gland will begin to enlarge markedly at puberty particularly in girls. This enlargement is considered as a compensatory mechanism to trap more iodine
- In some patients large goiter may cause pressure on the trachea and oesophagus which cause difficulty in breathing, irritative cough, voice changes and some time may affect swallowing

**Etiology of Iodine deficiency**

- Deficient intake diet and water in mountain areas, isolated localities depending on well or spring in which iodine content is low
- Increased requirement in developing fetus, newborn, young child, adolescents especially in female, pregnant and lactating women

- Intake of goitroenic substances occur naturally in food that acts by blocking absorption, utilization of iodine. They are found in cabbage, turnips, peanut and soya beans. Cooking inactivates these substances. Other goitrogens include sulfonamide
- Deficiency of enzymes needed in the metabolism of iodine

**Symptoms**[16]

Main symptoms includes

A swelling, ranging in size from a small nodule to a massive lump in the front of the neck just below your Adam's apple

- A feeling of tightness in the throat area
- Hoarseness
- Neck vein distention
- Dizziness when the arms are raised above the head
- Difficulty breathing (shortness of breath) coughing, wheezing (due to compression of the windpipe) are rare symptoms
- Difficulty swallowing (due to compression of the esophagus) is also rare

Other symptoms of goiters can include

- An increased resting pulse rate
- Rapid heartbeat
- Diarrhea, nausea and vomiting
- Sweating without exercise or increased room temperature
- Shaking
- Agitation

The six symptoms listed above are also symptoms of hyperthyroidism. Hyperthyroidism is a condition in which the thyroid is overactive. Some people with goiter may also have hyperthyroidism.

Other symptoms of goiters can includes

- Fatigue, constipation and dry skin
- Weight gain
- Menstrual irregularities

The three symptoms listed above are also symptoms of hypothyroidism. Hypothyroidism is a condition in which the thyroid is underactive. Some people with goiter may also have hypothyroidism

**Causes and Risk Factors for Goiter[17]**

A risk factor is something which increases the chances of developing a condition or disease. For example, a risk factor for heart disease is smoking if you smoke regularly your risk of developing heart disease is increased

Thyroid gland produces two main hormones- thyroxine $T_4$ and triiodothyronine ($T_3$). These hormones circulate in your bloodstream and help regulate your metabolism. They maintain the rate at which your body uses fats and carbohydrates, help control your body temperature, influence your heart rate, and help regulate the production of proteins. thyroid gland also produces calcitonin -a hormone that helps regulate the amount of calcium in blood. Thyroid gland also produces calcitonin -a hormone that helps regulate the amount of calcium in blood.

**Iodine deficiency**

Iodine deficiency is the major cause of goiter worldwide, but this is rarely a cause in more economically developed countries where iodine is routinely added to salt.As iodine is less commonly found in plants, vegan diets may lack sufficient iodine; this is less of a problem for vegans who live in countries such as the United States that add iodine to salt**.**

**Dietary iodine is found in**

- seafood
- plant food grown in iodine-rich soil
- cow's milk
- The thyroid gland needs iodine to manufacture thyroid hormones, which regulate the body's rate of metabolism.

**Autoimmune disease**

The main cause of goiter in developed countries is autoimmune disease. Women over the age of 40 are at greater risk of goiter, as are people with a family history of the condition.Hypothyroidism is the result of an underactive thyroid gland, and this causes goiter. Because the gland produces too little thyroid hormone, it is stimulated to produce more, leading to the swelling.This usually results from Hashimoto's thyroiditis, a condition in which the body's immune system attacks its own tissue and causes inflammation within the thyroid gland.

**Hyperthyroidism**

Hyperthyroidism - an overactive thyroid gland - is another cause of goiter; too much thyroid hormone is produced. This usually happens as a result of Graves' disease, an autoimmune disorder where the body's immunity turns on itself and attacks the thyroid gland, causing it to swell.

**Graves' disease**

A goiter can sometimes occur when your thyroid gland produces too much thyroid hormone (hyperthyroidism). In Graves' disease, antibodies produced by your immune system mistakenly attack your thyroid gland, causing it to produce excess thyroxine. This overstimulation causes the thyroid to swell.

**Pregnancy**

A hormone produced during pregnancy, human chorionic gonadotropin (HCG), may cause your thyroid gland to enlarge slightly.

**Risk factors of goiter[18]**

Goiters can affect anyone. They may be present at birth and occur at any time throughout life. Some common risk factors for goiters include:

- **nodules** - benign lumps
- **smoking** - thiocyanate in tobacco smoke interferes with iodine absorption
- **hormonal changes** - pregnancy, puberty, and menopause can affect thyroid function

- **Thyroiditis** - inflammation caused by infection.
- **Lithium** - a psychiatric drug that can interfere with thyroid function
- **Overconsumption of iodine** - too much iodine can cause a goiter
- **A lack of dietary iodine -** People living in areas where iodine is in short supply and who don't have access to iodine supplements are at high risk of goiters.
- **Being female -** Because women are more prone to thyroid disorders, they're also more likely to develop goiters.
- **Age -** Goiters are more common after age 40.

- **Medical history**- A personal or family history of autoimmune disease increases the risk.
- **Pregnancy and menopause -** For reasons that aren't entirely clear, thyroid problems are more likely to occur during pregnancy and menopause.
- **Certain medications -** Some medical treatments, including the heart drug amiodarone (Cordarone, Pacerone, others) and the psychiatric drug lithium (Lithobid, others), increase the risk.
- **Radiation exposure -** Your risk increases if you've had radiation treatments to neck or chest area or you've been exposed to radiation in a nuclear facility, test or accident.

**Diagnosis of Goiter[19]**

General practitioner and/or primary care physician may detect a swollen thyroid gland by feeling the patient's neck and asking him/her to swallow during a routine physical exam. Sometimes the nodules may also be detected simply by touch. A physical examination of the neck may also allow the doctor to assess the size of the thyroid gland and the extent of the swelling.

Examples of possible tests includes

- Hormone test
- Antibody test
- Ultrasound
- Thyroid scan (radioactive iodine scan)

**Treatment Options For Goiter[19]**

The type of treatment may depend on various factors, including the size of the thyroid gland, symptoms their severity and any underlying conditions. If the patient's

goiter is small, the thyroid gland is working properly and there are no underlying conditions the doctor will probably recommend long term monitoring but no treatment

**Hypothyroidism**

In cases caused by underactive thyroid or hypothyroidism, treatment is with a synthetic replacement of thyroid hormone.The dosage of synthetic thyroxine is gradually increased until measurements indicate normal thyroid function has been restored. Synthetic preparations of T4 (l-thyroxine) are preferred, but preparations of T3 (liothyronine) and combinations of both may be tried, as may desiccated animal thyroid extract.

**Hyperthyroidism**

In goiters caused by overactive thyroid or hyperthyroidism, treatment aims to counter the excess hormone production. For instance, anti-thyroid drugs such as thionamide drugs gradually reduce excess hormone levels. Radioactive iodine to decrease thyroid function and stop hormone production is also a treatment option for hyperthyroidism.

**Radioactive iodine**

This is a possible treatment option for patients with an overactive thyroid gland. The iodine is taken by orally. The radioactive iodine destroys thyroid cells when it reaches the thyroid gland resulting in a smaller goiter. The patient may end up with an under active thyroid gland and subsequently need hormone therapy.

**Iodine supplements**

The patient will be prescribed iodine supplements if the goiter is caused by an iodine deficiency. Iodine supplements are available over the counter (OTC). It is important to follow the dosage prescribed by your doctor.

**Surgery**

This is an option if the goiter is so large that the patient has problems in breathing or swallowing and other treatments have not worked. In most cases half the thyroid gland will be surgically removed. The surgeon will remove enough of the gland to relieve symptoms. Some patients, however will need hormone therapy after surgery. Possible complications from surgery includes

**Infection**

Nerve damage that affects the voice box that gives the patient a permanent hoarse voice and damage to the parathyroid glands, which regulate body calcium levels.

**Medications**

Thyroxine is a thyroid hormone replacement therapy. It is prescribed if the cause of

the goiter is an underactive thyroid (hypothyroidism). Other medications are prescribed if the cause of the goiter is an overactive thyroid (hyperthyroidism). These drugs include methimazole and propylthiouracil. Aspirin or a corticosteroid medication might be prescribed if the underlying cause of the goiter is inflammation. Small doses of iodine (in the form of Lugol's or potassium iodine solution) can be prescribed if the goiter is due to iodine deficiency

**Mechanism of action[19]**

Thyroxine penetrates cells and combines with a nuclear receptor. A specific DNA sequence called thyroid hormone response element has been identified in the regulatory region of specific genes to which the $T_3$ receptor complex binds to produce depression of gene transcription. This results in expression of predetermined genetically coded pattern of protein synthesis.

**Possible Complications of Goiter[20]**

While small goiters do not usually cause any problems large ones can make it hard for the patient to breathe and swallow properly, as well as causing a cough and hoarseness.

**Goiter:**[21] Enlargement of the thyroid gland, frequently due to excessive stimulation of hormone production. Large goiter can affect breathing and/or swallowing.

**Heart problems:**[22] Elevated thyroid hormones can cause tachycardia, atrial fibrillation and congestive heart failure.

**Osteoporosis:**[23] Long-term elevation of thyroid hormone levels can lead to osteoporosis via an inability to incorporate calcium into bones.

**Eye problems:**[24] patients with hyperthyroidism may develop eye disorders, including bulging, red or swollen eyes, sensitivity to light and visual disturbance.

**Skin problems:**[25] some patients with hyperthyroidism can develop dermopathy, causing redness and swelling, often on the shins and feet.

**Thyrotoxic crisis:**[26] hyperthyroidism carries a risk of thyrotoxic crisis - a sudden intensification of symptoms, leading to fever, tachycardia and even delirium.

## 2. LITERATURE SURVEY

- **Young RL *et. al.*, (1975)** reported thyroid-stimulating hormone levels in idiopathic goiter. TSH levels were significantly higher in patients with goiter less than 1 year compared to goiter greater than 1 yr ($P < 0.025$). In patients with goiter greater than 1 year the TSH levels remained significantly higher than normal ($P< 0.025$). These results support the hypothesis that TSH plays a role in the genesis of idiopathic goiter[27]

- **Detiuk ES *et. al.*, (1976)** reported antithyroid activity of various newly synthesized 1,3-thiazine derivatives. It was revealed that three newly synthesized 1,3-thiazine derivative (1,3-thiazandithion-2,4, 1,3-thiazanthion-2-on-4-thiosemicarbazone-4, and 1,3-thiazandion-2,4) possessed and antithyroidal action in doses equal to 15% of $LD_{50}$ in the two former preparations it was accompanied by an goiterogenic effect. 1,3-thiazandion-2,4 had the most antithyroidal action and relatively weak goiterogenic effect[28]

- **Lupulescu A *et. al.*, (1976)** reported Goiter formation following prostaglandin administration in rats. The present findings demonstrate that the chronic administration of prostaglandins exerts significant effects on thyroid gland and goiter formation (goitrogenesis), radio iodine metabolism, and hormone synthesis, and that these effects are mediated by TSH secretion[29]

- **Hitoshi Fukuda *et. al.*, (1980)** reported sequential changes in the pituitary thyroid axis during pregnancy and lactation in the rat. The results showed that after delivery, high plasma TSH and low thyroid hormone levels persisted throughout lactation and returned to normal only after weaning. In rats whose pups were removed shortly after delivery (nonlactating), these hormones returned to the normal ranges within a few days. $FT_4F$ became normal in both lactating and nonlactating rats after delivery, but the free $T_4$ concentration index was significantly lower in the former. There was a significant reduction of plasma $T_4$ in sucklings when their nursing mother was thyroidectomized. However, nearly normal levels of plasma $T_4$ were maintained in sucklings whose thyroidectomized mother was supplemented with a physiological replacement dose of $T_4$[30]

- **Murakami N *et. al.*, (1984)** reported that thyroid hormone maintains normal circadian rhythm of blood corticosterone levels in the rat by restoring the release and synthesis of ACTH after thyroidectomy. Three weeks after thyroidectomy, daily overall treatment with thyroxine ($T_4$) or 3,5,3'-triiodothyronine ($T_3$) completely restored the amplitude of the circadian adrenocortical rhythm to the previous level within 2 weeks. Thyroidectomy did not affect the circadian rhythm in water intake. However, thyroidectomy resulted in a loss of significant difference of plasma adreno corticotrophin (ACTH) levels between the morning and the evening, by decreasing the evening levels. Similarly, pituitary ACTH content was decreased by thyroidectomy. Replacement of $T_4$ completely restored the decreased ACTH levels to the previous ones. These results suggest that thyroid hormone plays an important role in maintenance of the normal amplitude in circadian adrenocortical rhythm in the rat, by affecting ACTH synthesis[31]

- **Rajeswary mageswaran *et. al.*, (1984)** studied Preliminary Studies on the Iodine Content of some Marine Algae from Coastal Areas of Jaffna Peninsu.These seaweeds could be exploited for the commercial extraction of iodine. Several other species have reasonable amounts of iodine and these could be used in the manufacture of fortified cattle teed and for preparation of high iodine food items for human consumption[32]

- **Michalkiewicz M *et. al.*, (1989)** reported alterations in thyroid blood flow induced by varying levels of iodine intake in the rat. The results showed that a slight decrease in plasma $T_4$ levels occurred over the 133 days of low iodine diet (LID) treatment; however, this dietary regimen did not alter circulating levels of $T_3$ or TSH or thyroidal vasoactive intestinal peptide (VIP) concentration. High iodine diet (HID) treatment had opposite effects, in general, to those of LID. Thyroid blood flows were decreased by 34%, 56%, 46%, and 35% after 3, 7, 14, and 133 days of treatment with HID, respectively. Circulating levels of $T_4$ were increased over the 133 days of HID treatment, whereas plasma levels of $T_3$ and TSH and thyroid weights remained unchanged from those in control rats over this period of study. A small decrease in thyroidal VIP concentrations coincident with the decrease in thyroid blood flow was observed at the beginning of the HID treatment.

Neither LID nor HID had any effect on blood pressure, cardiac output, or blood flow in other organs[33]

- **Taylor T *et. al.*, (1990)** performed thyroid hormone regulation of TRH mRNA levels in rat paraventricular nucleus of the hypothalamus changes during ontogeny. The results showed that in the euthyroid animals, TRH mRNA increased from E20 (150 ± 9 OD units) to 7 days (222 ± 5 OD units) and remained unchanged at 21 days (252 ± 27 OD units) and 56 days (244 ± 6 OD units)[34]

- **Pawlikowski M *et. al.*, (1998)** reported effects of octreotide on propylthiouracil induced goiter in rats. a quantitative evaluation. Goiter formation was assessed by measurement of the main histological compartments of the thyroid as well as by morphometric analysis of the vascularization and blood supply of the gland. Although treatment with octreotide did not prevent the goiter formation, it clearly reduced blood supply and vascularization of the thyroid and counteracted propylthiouracil induced increase in the relative volume of follicular epithelium. To conclude, the somatostatin analog octreotide is effective in reduction of goiter vascularisation. This finding provides a rationale for the clinical trials of the treatment of hypervascular goiter by somatostatin analogs[35]

- **Yoshihisa kato *et. al.*, (1998)** reported reduction of thyroid hormone levels by methylsulfonyl metabolites of tetra and penta chlorinated biphenyls in male sprague dawley rats. The results show that the tested 3- and 4-$MeSO_2$ metabolites of tetra and penta CBs reduce thyroid hormone levels in rats, suggesting that the metabolites may act as endocrine disrupters[36]

- **Nariaki Fujimoto *et. al.*, (1999)** reported changes in thyroid function during development of thyroid hyperplasia induced by kojic acid in F344 rats. The results showed that after 4 weeks, thyroid hyperplasia was apparent in males, associated with a decrease in $^{125}I$ uptake into the thyroid gland to only 3% of that in controls. The serum $T_3$ and $T_4$ levels dropped to 0.36 ng/ml, 1.7 µg/dl from the initial values of 0.61 ng/ml, 4.0 µg/dl and TSH increased seven times to 15 ng/ml. In females, the effects on thyroid weight and $^{125}I$ uptake were less prominent, although the changes in serum $T_3$, $T_4$ and TSH levels were similar to those in males[37]

- **Liu XM *et. al.*, (2001)** studied Anti-inflammatory and anti-ulcer activity of Calligonum comosum in rats. the tested extract has shown significant anti-ulcer and cytoprotective effects against gastric ulcers experimentally induced by non-steroidal anti-inflammatory drugs and necrotizing agents. Further studies are needed to elucidate the mode of action and better evaluate the potential therapeutic value in the treatment of C. comosum aerial parts[38]

- **Msuya E *et. al.*, (2002)** studied Ulva reticulata and GracilariacrassaMacroalgae That Can Biofilter Effluent from Tidal Fishponds in Tanzania. The algal biomass produced was of good quality with protein dry weight contents of 13% for G. crassa and 26 % for *U. reticulata*[39]

- **Choudhary GP *et. al.*, (2002)** studied Anti-ulcer activity of the ethanolic extract of *Terminalia belerica Roxb.* Phytochemical investigation suggested that several species of terminalia were found to exhibited anti-ulcer activity15 Phytochemical investigation of this medicinal plant revealed the presence of ellagic acid and gallic acid. Ellagic acid, a widely occurring polyphenol possesses strong antioxidant activity. It has a marked inhibitory effect on acid secretion and the occurrence of stress-induced gastric lesion, and these effects may be attributed to the inhibition of H+ and K+ ATPase activity[40]

- **Mildred S Christian *et. al.*, (2003)** performed evaluation of thyroid function in neonatal and adult rats. The neglected endocrine mode of action. The results indicate that in rodents, decreased serum levels of $T_3$ and $T_4$ and increased serum TSH levels, with sustained release of TSH and resultant follicular cell hypertrophy/hyperplasia, are typical hormonal and histopathological findings attributable to compounds altering thyroid function. Hypothyroidism early in the neonatal period can affect reproductive endpoints in both male and female rats, with the critical period of exposure being the first two weeks postnatal. Hypothyroidism has been shown to reduce gonadotrophin levels and delay pubertal sperm atogenesis in male rats and to block gonadotropin induced first ovulation in immature female rats by decreasing FSH and luteinizing hormone (LH) serum concentrations[41]

- **Mortoglou A *et. al.*, (2004)** reported the serum triiodothyronine to thyroxine ($T_3/T_4$) ratio in various thyroid disorders and after Levothyroxine replacement therapy. The values of $T_3/T_4$ ratio in the various categories were: Eu= 15.89, Hypo = 24.12, hyper= 19.57, hypoRx= 13.42, DQ= 15.16. The $T_3/T_4$ ratio was lower in the hypoRx group than in the EU group (P <0.001), although neither TSH values nor $T_3$ values showed any differences between these two groups, whereas $T_4$ levels were significantly higher in the hypoRx group (Eu= 7.99±1.46, hypoRx = 9.11±1.58, P< 0.001). The $T_3/T_4$ ratio in the DQ group was comparable to that of the Eu group, but significantly lower than the hyper group (P=0.95 between Eu and DQ, P<0.001 between DQ and hyper). Conclusions: These findings indicate that in hypothyroid patients, L-$T_4$-replacement that is sufficient to maintain a normal serum TSH is accompanied by a serum $T_4$ that is higher than in normal individuals and may not result in an appropriately normal serum $T_3$ concentration. In Thyrotoxicosis, a ratio of total $T_3/T_4$ >18.9 suggests Graves' disease or toxic multinodular goiter whereas $T_3/T_4$ <16 suggests thyroiditis (subacute or silent)[42]

- **Kuppusamy *et. al.*, (2004)** studied Copper removal from aqueous solution by marine green alga *Ulva reticulata*.The present work evaluated the removal of copper (II) from aqueous solution using *U. reticulata* biomass. The sorption capacity of copper on U. reticulate increased with increase in pH reaching a maximum at 5.5[43]

- **Rao V *et. al.*, (2004)** studied Antiulcer activity of Utleria salicifolia rhizome extract. The present study showed that the ethanolic extract of Utleria salicifolia possess gastroprotective activity as evi-denced by its significant inhibition in the formation of ulcers induced by various physical and chemical agents. Pylorus ligation-induced ulcers are due to autodigestion of the gas-tric mucosa and break down of the gastric mucosal barrier[44]

- **Telesphore Benoit Nguelefack *et. al.*, (2005)** studied Tthe Antiulcer effects of the methanol extract of the leaves of aspilia africana (asteraceae) in rats. The extract at the dose of 1 g/kg reduced gastric lesion in the pylorus ligated rats by 52 % although the gastric acidity remained higher as compared to the control. These

findings show that methanol extract of the leaves of A. africana possess potent antiulcer properties[45]

- **Pattama Ratana *et. al.*, (2006)** studied Nutritional Evaluation of Tropical Green Seaweeds *Caulerpa lentillifera* and *Ulva reticulate*.The results of the present study concluded that these seaweeds can provide dietary alternatives due to their nutritional values. Their commercial values can be enhanced by promoting the use in foods and expanding the range of seaweed-based product[46]

- **Sudipta Chakrabarti *et. al.*, (2007)** reported thyroid dysfunction modulates gluco regulatory mechanism in rat. The experimentally manipulated rats displayed higher levels of liver glycogen and serum glutamic pyruvic transaminase. Liver histology of hyperthyroid treated rats revealed hepatotoxicity. From the results it can be concluded that thyroid gland plays an important role in glucose homeostasis[47]

- **Malairajan *et. al.*, (2007)** studied Antiulcer activity of crude alcoholic extracts of bougainvillea spectabilis willd. The results demonstrated that ethanol extract of B.spectabilis produced antiulcerogenic effect in different ulcer models, thus suggesting that the extract possess antisecretory, cytoprotective and proton pump mechanism. Hence the B. spectabilis can be used as antiulcerogenic agent in prolonged aspirin therapy[48]

- **Satoko Gunji *et. al.*, (2007)** reported effects of extracts from tropical seaweeds on 2, 2-Diphenyl 1-Picryl Hydrazyl (DPPH) radicals and Caco-2, cells treated with hydrogen peroxide. Ethanol extracts were prepared from green algae. The ethanol extracts from *ulva reticulata* showed the strongest DPPH radical scavenging activity. These extracts also had the highest concentrations of total phenol and flavonoid. The ethanol extracts of the 6 Indonesian seaweeds decreased Caco-2, cell viability when such cells were treated with 600 µM hydrogen peroxide[49]

- **Yingheng Liu *et. al.*, (2008)** reported that serum thyroid hormone levels may not accurately reflect thyroid tissue levels and cardiac function in mild hypothyroidism. The results show that thyroid hormones are important regulators of cardiac function and myocardial arteriolar density[50]

- **Raju D *et. al.*, (2009)** studied Evaluation of Anti-ulcer activity of methanolic extract of Terminalia chebula fruits in experimental rats. This present study indicates that Terminalia chebula fruit extract have potential anti ulcer activity in the both models. These results may further suggest that methanolic extract was found to possess antiulcerogenic as well as ulcer healing properties, which might be due to its antisecretory activity[51]

- **Ghangale G *et. al.*, (2009)** studied Evaluation of Antiulcer Activity of Ocimum Sanctum in Rats. The present investigation revealed that Ocimum sanctum exhibited significant antiulcer activity by enhancing antioxidant potential of gastric mucosa thereby reducing mucosal damage[52]

- **Moumita Mukherjee *et. al.*, (2010)** studied Anti-ulcer and antioxidant activity of GutGard. The present study revealed that the GutGard™ was a significant inhibitor of gastric mucosal lesions caused by pylorus ligation, cold-restraint stress and indomethacin in rats thereby confirming its anti-ulcerogenic activity. The cytoprotective effect could be partially due to flavonoid content of GutGard™ and its reactive oxygen species scavenging property[53]

- **Samuel A *et. al.*, (2010)** studied Anti-ulcer & antioxidant activities of Hedranthera barteri (Hook F.) Pichon with possible involvement of H+, K+ ATPase inhibitory activity. the present results showed a significant anti-ulcer potential of DMHBR with more of cytoprotective than anti-secretory properties. It exhibited a proton pump inhibition and its anti-ulcer properties may be partly ascribed to its antioxidant activities. Further laboratory work on the separate phytochemical constituents of DMHBR will suggest the actual compound responsible for its anti-ulcer properties,especially, its proton pump inhibitory activity[54]

- **Kolanjinathan K *et. al.*, (2011)** studied Comparitive Studies on Antimicrobial Activity of *Ulva reticulata and Ulva lactuca* against Human Pathogens.The study of antimicrobial activity of seaweeds *Ulva reticulate and Ulva lactuca* extracts showed promising antimicrobial activity against bacterial and fungal human pathogens[55]

- **Karolin Kamel Abdul-Aziz *et. al.*, (2011)** studied Comparative Evaluation of the Anti-ulcer Activity of Curcumin and Omeprazole during the Acute Phase of Gastric Ulcer. Curcumin exerts its anti-ulcer activity not only by af-fecting oxidative stress and total antioxidant capacity but also by inhibiting IL-6 secretion and preventing apop-tosis. Furthermore, curcumin promotes gastric ulcer pre-vention/healing by induction of angiogenesis in the granular tissue of ulcers. Further prospective studies are required to investigate the mechanism underlying the upregulation effect of curcumin on VEGF signalling pathways[56]

- **Venkat Rao N *et. al.*, (2011)** studied Evaluation of anti -ulcer activity of momordica charantia in rats. Fruit extracts of M.charantia exhibited a significant anti-arthritic and anti-ulcer activities in experimental animals rats/mice.Alcoholic extract exhibited relatively better anti-arthritic and anti-ulcer activities than aqueous extract. The difference in the evaluated activities could be due to the number /quantity of phytoconstituents present in these extracts[57]

- **Issi M *et. al.*, (2011)** reported the effect of classical Theileriosis treatment on thyroid hormone levels in cattle naturally infected with *Theileria annulata*. It was detected that mean values of free $T_3$, free $T_4$, total $T_3$ and $T_4$ of thyroid hormones were significantly lower the values of the control group in pre treatment period ($p<0.001$, $p<0.01$, $p<0.001$ and $p<0.01$, respectively), mean value of the TSH increased ($p<0.05$). In post-treatment analysis, there was not statistical difference in all thyroid hormones except for total $T_4$ concentration. It was established that although there was increase in total $T_4$ level when compared with pre-treatment values, the difference between control group and the groups was low on the level of $p<0.05$[58]

- **Seyed Fazel Nabavi *et. al.*, (2011)** reported protective effect of curcumin and quercetin on thyroid function in sodium fluoride intoxicated rats. The results shown that pretreatment especially with the higher dosages of curcumin and quercetin, and also vitamin C prior to fluoride exposure effectively kept the serum thyroid hormone levels near the normal range[59]

- **Jiashu Yu *et. al.*, (2011)** reported that vitamin E ameliorates iodine induced cytotoxicity in thyroid. The results showed that excess iodine leads to thyroid

damage and vitamin E supplementation can partly ameliorate iodine induced thyroid cytotoxicity[60]

- **Felipe Meira de-Fariaa *et. al.*, (2012)** studied Mechanisms of action underlying the gastric antiulcer activity of the Rhizophora mangle L.Our study on the pharmacological mechanisms involved in the antiulcer activity of Rhizophora mangle reinforces its traditional medicinal use. The treatment did not show any toxic effects, but instead, demonstrated to have cytoprotective, antisecretory and healing properties, possibly, due to the stimulation of PGE2 by the upregulation of COX-2. Considering the currenttherapies are based on the use of antisecretory or cytoprotective drugs, the Rhizophora mangle arises as a promising alternative antiulcer therapy[61]

- **Pragyandip Dash *et. al.*, (2012)** studied Anti-Ulcer Activity of Methanolic Extract of *Haldina cordifolia*.The present study showed that pretreatment with the leaf extract (both hot water and cold water) of *Haldina cordifolia* caused a beneficial effect on NSAID- induced gastric ulcer in rats as evidenced by the reduction in the ulcer score[62]

- **Vandana Panda *et. al.*, (2012)** studied Anti-ulcer activity of Ipomoea batatas tubers (sweet potato). It has been proven already by many scientific studies that antioxidants have ulcer healing properties. In reference to this, we tried assessing the ulcer healing effect of Ipomoea batatas tubers[63]

- **Prasanth Reddy V *et. al.*, (2012)** studied Evaluation of Anti-Ulcer Activity of Citrullus Colocynthis Fruit Against Pylorus Ligation Induced Ulcers in Male Wistar Rats. It showed also significant ($P<0.001$) decrease in number of ulcers and ulcer score index in pylorus ligation ulceration model. In conclusion the antiulcer properties of the extracts may be attributed to the presence of phytochemicals like flavanoids, saponins, alkaloids and tannins present in the plant extract with various biological activities[64]

- **Dilpreet Kaur.A *et.al*., (2012)** studied Herbal Drugs with Anti Ulcer Activity. In this review attempts have been made to know about some plants which may be used in treatment or prevention of peptic ulcers. Various plants like Cynodon dactylon, Ocimum sanctum, Glycyrrhiza glabra, Ficus religiosa proved active in antiulcer therapy[65]

- **Elango *et. al.*, (2012)** Studied Antiulcer activity of the Leaf ethanolic extract of *Mimosa pudica* in Rats. Ethanolic extract of *Mimosa pudica* (MP) reduced ulcer incidence, when compared to the control as evident by decrease in ulcer score in all the three models. Anti-secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was decrease in gastric volume and reduction in free and total acidity in the treated with ethanolic extract. This indicates that the leaf extracts of *Mimosa pudica* has antiulcer activity[66]

- **Hemamalini *et. al.*, (2012)** studied Evaluation of anti-ulcer activity of methanolic extracts of *Kigelia africana, Sophora interrupta and Holoptelea integrifolia* leaves in experimental rats. Leaf extracts of *K.africana, S.interrupta, H.integrifolia* exhibited a significant anti-ulcer activities in experimental animals rats. Methanolic extract of *Holoptelea integrifolia* exhibited relatively better anti-ulcer activities than *K.africana and S.interrupta* extracts. The difference in the evaluated activities could be due to the number and the quantity of phytoconstituents present in the extracts[67]

- **Lavanya A *et. al.*, (2012)** studied Antiulcer activity of *Canavalia virosa* (roxb) w&a leaves in animal model. From this study, it is clearly evident that *Canavalia virosa* (Kozhi Avarai Ilai) Chooranam has significant action as anti-ulcer activity in animal models at the dose level of 200mg/kg-1. But it has no muco protective activity and moderate gastric anti-secretary when compared with that of reference drug[68]

- **Felix S *et. al.*, (2012)** observed Lactic acid fermentation of seaweed (*ulva reticulata*) for preparing Marine Single Cell Detritus (MSCD). The process of fermentation was monitored continuously by estimating the lactic acid concentration, pH, and the odour. The result established that The microbial

propagation pattern for a period of 50 days also has been observed to understand the products keeping quality[69]

- **Haqeeq Ahmad *et. al.*, (2013)** Studied Evaluation of Anti-ulcer activity of hydro alcoholic extract of Post Sumaq (Rhus coriaria Linn.) in Ethanol induced Gastric ulcer in experimental Rats. According to Unani physicians the causes of gastric ulcer are Khilte Haad (Irritant and corrosive humour) fuzlat (Wastematerials which accumulate in the stomach and get infected), Nawazil (Descendants which get purulent), intake of hot and spicy foods, excessive use of alcohol, prolong stress and strain, and chronic gastritis and indigestion. Additionally, Mizaj (temperament) of drugs and diseases is an important concept of Unani medicine kept in mind when treating a disease and usually drug of opposite Mizaj to disease is used[70]

- **Pradhan D *et.al.*, (2013)** studied Anti-ulcerogenic activity of Ethanolic Extract of Cucumis sativus L. against NSAID (Aspirin) induced Gastric Ulcer in wistar albino rats. The current study showed that ulcer index in the group 5 received 400 mg/kg body weight of the ethanolic extracts of cucumber showed a significant less ulcer index when compared to control. Aspirin causes mucosal damage by interfering with prostaglandin synthesis,increasing acid secretion and back diffusion of H+ ion. Overall, ethanolic extract of 400mg/kg body weight has shown a substantial and significant protection against gastric ulcers in all the models. The ulceroprective activity of cucumber may be backed by presence of alkaloids, steroids, flavonoid, polyphenols have been proven reduction of gastric acid volume, free acidity, total acidity, antioxidant, anti-inflammatory and immunimodulatory activity[71]

- **Gindi Sumalatha *et. al.*, (2013)** Studied Evaluation of antiulcer activity of *wedelia calendulacea* aqueous extract in rodents. The present study confirms the antiulcer activity of WC as it produced significant antiulcer property by their antisecretory ,cytoprotective and proton pump inhibitory properties. Further studies are needed to isolate the chemical moiety responsible for the antiulcer activity of this extract[72]

- **Kamble Rahul Devidas *et. al.*, (2013)** studied A Review on Antiulcer Medicinal Plants. Medicinal plants provide an effective and safer way in disease management. Many medicinal plants exhibit antiulcer activity and found useful in the treatment of peptic ulcer. In this review attempts have been made to know about some plants which may be used in treatment or prevention of peptic ulcer. Various plants like Nerium indicum, Ocimum sanctum, Argyreia speciosa, Bauhinia purpurea, Benincasa hispida and Croton zambesicus proved active in antiulcer therapy[73]

- **Gehan Salah Eldin *et. al.*, (2013)** studied Evaluation of Antiulcer Activity of red pepper and garlic bulb against Indomethacin- induced gastric ulcer in rats: biochemical and histopathological study. Conclusion, overall, the attenuation of gastric affronts of indomethacin by administration of fresh juices of red Pepper and garlic bulb regimen is indicative of their excellent gastroprotective and antioxidative potentials in rats. Efforts are ongoing to investigate the exact antiulcerogenic principles in these test plants and also harness their possible synergistic efficacy against gastric ulcer[74]

- **Sorabh Kumar Agrawal *et. al.*, (2013)** studied Evaluation of Anti Ulcer Activity of *Oldenlandia Corymbosa* (L). present study Aspirin was selected as ulcer producing agents . Aspirin is one of the most common to produce ulcer then present study demonstrated as alcoholic extract exhibited significantly dose depended anti ulcer activity. In comparison to alcoholic extract,aqueous extract show less no. of ulcers in stomach induced by Aspirin. The all histological changes absorbed in correlation with the physical, biochemical, and functional parameters of the stomach. It can be concluded that *Oldenlandia Corymbosa* (L) extracts possess a protective effect against Aspirin induced ulcer in rat, as evidenced by the physical, biochemical, functional and histological parameters[75]

- **Mahathi K *et. al.*, (2013)** studied Evaluation of Anti ulcer activity of methanolic extract of leaves of *Catharanthus Roseus* in experimental rats. The methanolic extracts of leaves of *Catharanthus roseus* showed significant, graded and dose dependent anti-ulcer activity in Forced swim induced ulcer in rats. In Forced swim induced gastric ulcer model, the methanolic extracts of leaves of *Catharanthus roseus* at doses of 250mg/kg and 500mg/kg P.O were found to be having

significant, graded and dose dependent Anti-ulcer activity and Anti-secretory activity when compared to control group using Ranitidine 5mg/kg as Standard. Thus from the present study it can be concluded that the methanolic extracts of leaves of *Catheranthus roseus* has Anti ulcerogenic and Anti-secretory in Forced swim induced ulceration in rats[76]

- **Saini V *et. al.*, (2013)** studied Antiulcer activity of pantoprazole from multiple-unit tablet dosage form. In the present study, modification of the original method described by kulkarni was used.The Anti-ulcer activity of alcohol induced ulcer rat model with 4 mg / kg b.w. of pantoprazole multiple-unit tablet showed that the tablets are able to protect ulcer formation by alcohol[77]

- **Ganapathy Selvam *et. al.*, (2013)** studied Developmental changes in the germination, growth and chlorophyllase activity of *vigna mungo* using seaweed extract of *ulva reticulata Forsskal*.The extract of *Ulva reticulate* found to have promising result by possessing fertilizer activity to enhance the germination and growth of *Vigna mungo*. Hence, this simple practice of application of ecofriendly seaweed liquid fertilizers to pulses is recommended to the growers for attaining better germination, growth and yield[78]

- **Mahadevan G *et. al.*, (2013)** studied Antifouling activity of the green seaweed Ulva reticulate and its epiphytic bacterial strains against marine biofilm bacteria.In natural concentration, the crude extracts of *Ulva reticulate* showed good inhibitory activity against all biofim forming bacteria[79]

- **Ingale Anand M *et. al.*, (2014)** studied A Comparative evaluation of the anti-ulcer activity of the extracts of seed and skin of *vitis vinifera* (grape) in wistar albino rats. The study shows that the ethanolic extract of grape seed has potential antiulcer activity than that of grape skin extract.the extract with the dose of 200mg/kg shows maximum anti-ulcer activity. Establishment of the antiulcer activity of GSE may provide a newer and economically better modality of treatment for peptic ulcer which may have a better safety and efficacy[80]

- **Ezekwesili Chinwe *et. al.*, (2014)** studied Evaluation of the anti-ulcer property of aqueous extract of unripe Musa paradisiaca Linn. peel in Wistar rats. Findings from our studies indicate that the aqueous peel extract of M. paradisiaca Linn. protected against ulcerative lesions induced by ethanol, aspirin and pyloric ligation. Similar to the action of cimetidine standard, the extract exacerbated indomethacin-induced ulcer. M. paradisiaca peel extract mimics the actions of cimetidines in ameliorating ulcers in all the experimental models adopted, therefore it may possess an antisecretory property[81]

- **Jaliwala *et. al.*, (2014)** studied Antiulcer and Anti-inflammatory activity of Cajanus Cajan linn. The results of present study show that the ethanolic extract of C. cajan Linn. Leaves extract possesses gastroprotective effect and improve ulcer-healing activity. Ethanolic extract also show possible free-radical scavenging property on endogenous PGs. Significant antiinflammatory effect was shown by the ethanolic extract on carrageenan-induced edema and cotton pellet granuloma in rats[82]

- **John Peter Paul J *et. al.*, (2014)** studied Seasonal variability of Ulva species (Green seaweed) in Tirunelveli region, the south east coast of Tamil Nadu, India.The present study was concluded that all the Ulva species (Chlorophyceae) exhibited the maximum frequency and density during the summer season followed by the declined trend was observed in the successive seasons. During the post monsoon season the frequency and density of Ulva species (Chlorophyceae) was minimum in the selected region of south east coast of Tamil Nadu[83]

- **Saraniya devi J *et. al.*, (2014)** studied Antimicrobial potential of silver nanoparticles synthesized using *Ulva Reticulata*.The study is to evaluate the antibacterial and antifungal activity of synthesized AgNPs. Antibacterial activity was tested against Gram positive Staphylococcus aureus, and Gram negative Escherichia coli Pseudomonas aeruginosa Bacillus sp.Klebsiella pneumoniae Antifungal activity was tested against Candida albicans Candida parapsilosis And Aspergillus niger[84]

- **John Peter Paul *et. al.*, (2014)** studied Distribution and seasonal variation of ulva species (green seaweed) in thoothukudi region, the south east coast of tamil nadu, india.From the present study, it was concluded that all the Ulva species (Chlorophyceae) exhibited the maximum frequency and density during the summer season followed by the declined trend was observed in the successive seasons[85]

- **Leelavathi MS *et. al.*, (2014)** studied Evaluation of Antioxidant properties of Marine Sea Weed samples by DPPH method. In methanol extract *Cymodeace rotundata, Gracillaria crassa* and *Cymodeace serrulata* showed the highest total antioxidant activity of compared with other samples. Ulva lactuca exhibited the highest antioxidant and free radical scavenging activities in petroleum ether extract[86]

- **Sumayaa S *et. al.*, (2015)** studied Preparation of novel seaweed recipes and standardisation for the human consumption.. From these studies we recommend the preparation of various recipes by using these seaweeds contains no toxicity[87]

- **Leelavathi MS *et. al.*, (2015)** studied Comparitive analysis of Phytochemical compounds of Marine algae isolated from Gulf of Mannar. The present study suggests that the seaweed extracts possessed phytochemical activity thus supporting their folkloric usage, promising a future scope for the use of these marine seaweeds against microbial populations[88]

- **Pranjit Santonu Bhajoni *et. al.*, (2016)** studied Evaluation of the Antiulcer Activity of the Leaves of *Azadirachta indica*: An Experimental Study. In conclusion, the leaves of *A. Indica* possess antiulcer activity and possibly act via multiple mechanisms including inhibition of the histamine-2 receptors/H + -K + -ATPase, prostaglandin modulation, or antioxidation. The present study confirms the folkloric claim of *A. Indica* being effective in the treatment of PUD[89]

- **Sumia Fatima *et. al.*, (2016)** studied Evaluation of Anti-Ulcer Activity of 70% Hydro-Ethanolic leaf extract of *Argemone mexicana* Linn. in Experimental Rats. The effect of anti-ulcer activity of 70% Hydro- Ethanolic leaf extract of *Argemone mexicana* Linn. seems to be effective and significant at a dose of 400mg/kg b.w.

The presence of flavonoids which have astringent property could be responsible for the anti-ulcer activity, and is more likely to be involved in the reaction with the proteins of the layer tissues and thereby showing the activity[90]

- **Chandrasekaran *et. al.*, (2016)** studied Phytochemical Analysis and Antifungal activity of Ulva Species from the Kanniyakumari Gulf of Mannar, South Coast India. The ethyl acetate extracts of *U. lactuca U. fasciata and U. reticulate* showed the presence of phytochemicals such as terpenoids, tannins and phenolic compounds strongly than the other extracts. The finding suggested that ethyl acetate extracts of *U. lactuca U. fasciata And U. reticulate* exhibited an antifungal substance for the treatment[91]

- **Abirami S *et. al.*, (2016)** studied Profiling of Omega 3 fatty acids from marine green algae *Ulva reticulata* and *Caulerpa racemosa*.The present study concluded that *C.racemosa* contains higher amount of omega 3-fatty acids than *U.reticulata*.These seaweeds could potentially be used, after processing, as a food. Further studies are needed on the nutritional and toxicological aspects of seaweed utilization as food and feed resources for human and animal consumption, respectively[92]

- **Rajakumar R *et. al.*, (2016)** studied Phytochemical analysis and biochemical estimation of *ulva lactuca and ulva reticulata*. The preliminary phytochemical performed by Harborne method and biochemical identication based on the standard methods. The results of the phytochemical analysis we identified Alkaloids, Phenol, Flavonoids, Sponin, Quinone, Steriods, Tanin, Carboxylic acid, Xanthoprotein, curcumins in both two seaweeds *U. lactuca* and *Ulva reticulata*[93]

- **Sundaram Ravikumar *et. al.*, (2016)** studied Antibacterial activity of *Ulva reticulata* from southwest coast of Kanyakumari, India.The study states that the n-butanolic extract of the seaweed powder of Ulva reticulate exerted notable antibacterial activity against tested bacterial strains. The maximum antibacterial activity was exhibited against Escherichia coli and Bacillus cereus in all concentrations[94]

- **Sharareh Khodami *et.al*., (2016)** studied Uptake of metals by live green macroalgae Ulva reticulate in industrial wastewater of Bayan Lepas, Penang, Malaysia.This study was conducted to investigate the ability of *Ulva reticulate* (Chlorophyta) to remove Cd, Co, Cr, Cu, Fe, Mn, Mg, Pb,V, and Zn from industrial waste water[95]

# 3. OBJECTIVE OF THE WORK

Marine natural products have been the focus of discovery for new products of chemical and pharmacological interest. The marine environment is a rich source of both biological and chemical diversity. This diversity has been the source of unique chemical compounds with the potential for industrial development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, fine chemicals and agrochemicals.In recent years,a significant number of novel metabolites with potent pharmacological properties have been discovered from the marine world

The *ulva* species is used as a traditional medicine for antibacterial, antifungal, goiter, gout, scrofula, burns, and antiulcer activity.

The objective of the current study is to evaluate the following

- To extract the active principle
- To perform Phytochemical screening

**Analytical determination**

- Fourier Transform Infrared Spectrophotometer
- HPTLC analysis

***In vivo* studies**

- To determine acute oral toxicity studies
  - Acute oral toxicity: Guideline number-423
- To screen the antiulcer activity
  - Ethanol induced gastric ulcer in rats
- To evaluate the antigoiterogenic activity
  - Propyl thiouracil induced goiter in rats

***In vitro* studies**

- To study the antioxidant activity
  - Diphenyl Picryl Hydrazyl (DPPH) scavenging method
- To find out the antimicrobial activity
  - Disc Diffusion method

# 4. SEAWEED PROFILE

## Seaweed Authentication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03.07.2017

Date.....

**CERTIFICATE**

This is certify that the marine alga (green) was collected from Mandapam coast, Tamil Nadu by **Mr. D. Kalivarathan (Reg. No: 261525151)** is identified as *Ulva reticulata* **Forsskal** belonging to the Division - Chlorophyta, Class – Chlorophyceae, Order – Ulvales and Family – Ulvaceae.

**(P. ANANTHARAMAN)**

**Seaweed *Ulva reticulata*[96]**

This is a small genus of marine and backish water green algae .it is edible and is often called 'sea lettuce'.species with hollow, one layered thalli were formerly included in enteromorpha ,but it is widely accepted now that such species should be included in ulva.

**Table No. 1: Scientific classification of *Ulva reticulata***

| | |
|---|---|
| Empire | Eukaryota |
| Kingdom | Plantae |
| Phylum | Chlorophyta |
| Class | Chlorophyceae |
| Order | Ulvales |
| Family | Ulvaceae |
| Genus | *Ulva* |
| Species | *Reticulate* |

**Taxonomy[96]**

*U. reticulata* belongs to the cosmopolitan genus *Ulva*, one of the first seaweed genera described by Linneaus in 1753, which includes species with foliose and tubular thalli. The genus *Ulva* is distinguished morphologically from closely related ulvalean *Enteromorpha* by its flat, two-cell layer (distromatic) morphology,in contrast to the latter with its tubular, single-cell (monostromatic) structure. However, *Ulva* and *Enteromorpha* are, as first thought by Linnaeus, not distinct genera after all, based on ITS nrDNA analysis

The plant is attached to a substratum throughout its life by hold fast, holdfast is a disc formed from primary cells of elongated, compact and strong nature; the thallus is an expanded sheet, 2 layered in thickness. it is a net like, pale green in colour and smooth delicate in texture; it is flat with numerous lacunae. the diameter of lacunae ranges from 0.4 to 7.0 cm. Cells in surface view re irregularly placed polygonal in shape, arranged with their long axis at right angle to the surface of the thallus ranging from 12.4 - 18.6 µ to 24.8 - 27.9 µ thallus grows parallel to the substratum and grows upto 62.4 cm to 187.2 cm in

length and 10 to 20 cm in width; the layers of the cells dilate in some parts of thallus and functions as air bladder; the marginal cell walls are stretched forming elongated marginal strands. these marginal strends give additional strength to the air bladder and avoid bursting very frequently.

T.S. of the thallus, the two layers of the cells separated by a cavity are equal in height with cells taller than the broad; the cells are arranged in two layers with distinct vesicular cavity and are covered with the thick cuticle measuring 9.3 to 12.4 µ towards the surface; each cell contains single parietal chloroplast; often with deeply incised or lobed margin containing a single pyrenoid; chloroplast is mostly located on the outer side of the cell while nucleus lies adjacent to the inner wall.

**Distribution**[97]

Algaebase gives the centre of distribution for *U. reticulata* as the Indo - west Pacific region. It is found in southeast Asia (Indonesia, Malaysia, the Philippines, Singapore, and Vietnam), Eastern Indian Ocean (Andaman and Nicobar Islands), Southwest Asia (Bahrain, India, Kuwait, Pakistan, Persian Gulf, Saudi Arabia, Sri Lanka), Western Indian Ocean (Kenya, Tanzania, Madagascar islands, Somalia, and Mauritius), Northern Indian Ocean (southern Red Sea, Eritea, Egypt, eastern Saudi Arabia), East Asia (southern Japan and Korea), Mid-Pacific Ocean (Hawaiian Islands), and in Oceania (Papua New Guinea, north Australia)

Westward, *U. reticulata* was reported in the Venezuelan waters of the Atlantic Ocean. With the assumption that the Indo-west Pacific region is its centre of distribution, it must have crossed the Atlantic via the Mediterranean Seas, before it reached the Venezuelan waters and become one of the exotic species in that area including as an associated species with mangrove *Rhizophora mangle*. Eastward, the species was reported to have also occurred in Chile as early as the 1950s . Assuming that a Pacific stock successfully crossed and reached the eastern Pacific, through Hawaii, there is, however, a disjunct in its distribution as there is so far no report of this species from any of the South Pacific island countries.

**Biology**[97]

The sporophyte (spore-producing) generation of *U. reticulata* produces containers called sporangia which release haploid, quadriflagellated zoospores through meiosis at the

middle portion of a mature thallus.When released,the zoospores leave the reticulated perforated thallus typical of *U. reticulata*. The released spores directly germinate, first, into a filamentous germling, and then into foliose gametophytes. Gametophytes in turn produce another container called gametangia in the mature thallus which releases haploid, biflagellated gametes through mitosis.

Gametes are isomorphic (isogametes) hence there is no distinct male or female rather they pair to form quadriflagellated zygotes at fertilization, developing into a filamentous germling, then into a perforated, foliose thallus. Unmated gametes are typically capable of functioning as asexual reproductive cells, attaching to substrates and growing into new (haploid) multicellular gametophytes

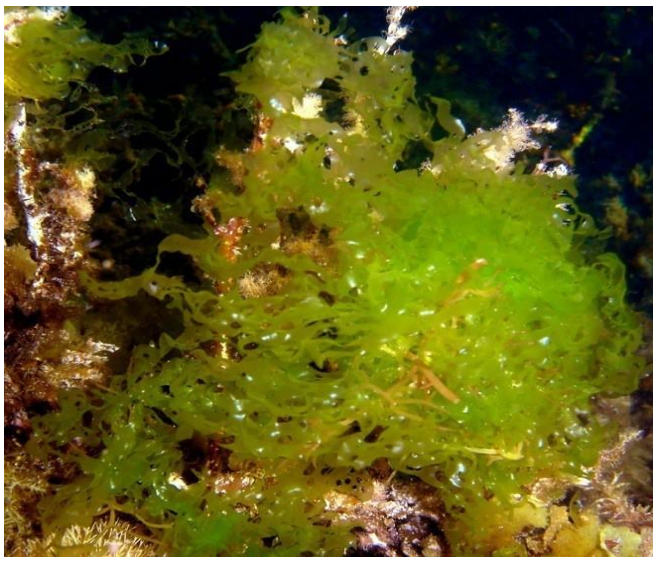
**Ecology**[97]

*U. reticulata* is an established tropical species that thrives well in clear, shallow waters. It requires warm water temperature of between 25 and 30°C, with growth occurring at a faster rate when inorganic nutrients, especially ammonia and phosphorous, are high due to its efficient nutrient uptake ability. It is likely that *U. reticulata* after macroalgal blooms alternates in occurrence with other species of algae that are common in eutrophied waters. In Mactan Island (Cebu, Philippines), for instance,the species has been found to be commonly insuccession with *Enteromorpha intestinales* and *E. clathrata* .

*U. reticulata* is conspicuously present during peak growth in protected marine habitats, estuaries, bays and lagoons, where salinity is around 34-35 ppt. The only exception is the Red Sea where salinities as high as 36.5 to 39 ppt seems to be at the extreme end of tolerance for *U. reticulata*.

Generally, the geographical distribution of *U. reticulata* is influenced mainly by water temperature. It can be found at quite a wide range of temperatures, from as low as 20 to 30°C to as high as 38°C in the Southern part of the Red Sea (Eritrea) during mid-summer . In Sri Lanka (Indian Ocean), surface water temperature all around the island is between 26 and 28°C while that in Southeast Asia it could range between 20 and 29°C. Vietnam which is connected to continental Asia, has a surface water temperature in winter of 20-23°C in its northern region and 25-29°C in the southern part, while in Thailand, Malaysia and the Philippines, temperature is normally about 28-30°C

**Fig. No. 3*:* Seaweed of *Ulva reticulata***



**Uses[97]**

**Economic Value**

The potential benefit of *U. reticulata* as human food is related to some findings that show high calorific (2828-3725 cal/g) and protein contents based on studies in India and in Thailand, respectively. In Japan, Philippines and Indonesia, *Ulva* is utilized as food in the form of fresh salad or used as ingredients in various food preparations

**Animal feed, fodder, forage**

- Bait/attractant
- Fishmeal
- Fodder/animal feed
- Forage
- Invertebrate food

**Fuels**

- Biofuels

**Human food and beverage**

- Emergency (famine) food
- Flour/starch
- Food additive
- Spices and culinary herbs
- Vegetable

**Materials**

- Chemicals
- Fertilizer
- Green manure
- Lipids
- Pesticide

**Medicinal, pharmaceutical**

- Source of medicine/pharmaceutical

# 5. METHODOLOGY

## Extraction

Extraction is defined as the process of isolation of material from an insoluble residue which may be liquid or solid, by treatment with a solvent on the basis of the physical nature of crude drug to be extracted, that is liquid or solid.

## Soxhlet extractor[98]

Soxhlet extractor is not limited to the extraction of lipids. Typically, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.

Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser

The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

**Fig. No. 4: Soxhlet extractor**

**Extraction of plant material**

Whole plant of *Ulva reticulate* were collected, dried material was ground into coarse powder which was used for further study to extract with various polar solvents using soxhlet apparatus.

**Petroleum ether extract**

About 500gm of dried coarse powder was extracted with 2.5 litre of petroleum ether by (60-80°C) continuous hot percolation using soxhlet apparatus. The extraction was continued for 24 hours. After completion of extraction, the petroleum ether extract was filtered and solvent was removed by distillation under reduced pressure. A dark green coloured residue was obtained. Then the extract was stored in a dessicator.

**Chloroform extract**

Marc obtained from the above extract was dried and extracted with 2.5 litre of ethanol. The extraction was continued for 24 hours. After completion of extraction, the extract was filtered and solvent was removed by distillation under reduced pressure. A green colour residue was obtained. Then it was stored in dessicator.

**Ethanolic Extract**

Marc obtained from the above extract was dried and finally extracted with 2.5 litre of ethanol. The extraction was continued for 24 hours. After completion of extraction, the extract was filtered and solvent was removed by distillation under reduced pressure. A green colour residue was obtained. Then it was stored in dessicator.

**Table No. 2: Identification of Plant Constituents by Preliminary Phytochemical Tests[99]**

| Test for Carbohydrate | | | |
|---|---|---|---|
| **S.No** | **Test** | **Observation** | **Inference** |
| 1. | **Molish's test**<br>The filtrate was treated with α-napthol solution added concentrated $H_2SO_4$ from sides of the tube | Violet ring at the junction of two liqueds | Presence of carbohydrates |
| 2. | **Fehling's test**<br>1ml Fehling's A & Fehling's B solutions was mixed and boiled for one minute. Add equal volume of test extracts . Heated in boiling bath for 5-10min. | A yellow, then brick red precipitate | Presence of reducing sugars |
| 3. | **Benedict's test**<br>Equal volume of Benedict's reagent and The minimum amount of extracts in test tube were mixed. Heated in boiling water for 5min. | Solution may appear yellow, green or red | Presence of reducing sugar |
| 4. | **Tannic acid test**<br>Mix 20% tannic acid with The minimum amount of extracts | White precipitate | Presence of starch |

| Test for protein | | | |
|---|---|---|---|
| **S.No** | **Test** | **Observation** | **Inference** |
| 1. | **Biuret test**<br>The minimum amount of extracts add 4% of NaOH and few drops of 1% $CuSO_4$ solution | Violet or pink colour | Presence of protein |
| 2. | **Xanthoprotein test**<br>The minimum amount of extracts, add 1ml of Conc. $H_2SO_4$ | White precipitate | Presence of protein |
| 3. | **Millon's test**<br>The minimum amount of extracts add 5ml of Millon's reagent | White precipitate on warming turns brick red | Presence of protein |
| **Test for Alkaloids** | | | |
| 1. | **Dragendroff's test**<br>The minimum amount of extracts, add few drops Dragendroff's reagent | Orange brown precipitate is formed | Presence of Alkaloids |
| 2. | **Mayer's test**<br>The minimum amount of extracts, add few drops of Mayer's reagent | Formation of precipitate | Presence of Alkaloids |
| 3. | **Hager's test**<br>The minimum amount of extracts, add few drops of Hager's reagent | Yellow precipitate | Presence of Alkaloids |

| Test for Steroid | | | |
|---|---|---|---|
| **S.No** | **Test** | **Observation** | **Inference** |
| 1. | **Salkowski reaction**<br>The minimum amount of extracts, add 2ml chloroform and 2ml Conc. $H_2SO_4$, shake well | Chloroform layer appears red and acid layer shows greenish yellow fluorescence | Presence of Steroid |
| 2. | **Liebermann's test**<br>The minimum amount of extracts with 3ml acetic acid anhydride. Heat and cool. Add few drops of Conc. $H_2SO_4$ | Blue colour appears | Presence of Steroid |
| **Test for Flavanoids** | | | |
| 1. | **Shinoda test**<br>The minimum amount of extracts, add 5ml 95% ethanol, few drops of Conc. HCl and 0.5g Magnesium turnings | Pink colour observed | Presence of Flavanoids |
| 2. | The minimum amount of extracts add lead acetate solution | Yellow coloured precipitate is formed | Presence of Flavanoids |
| **Test for Glycosides** | | | |
| 1. | **Legal's test**<br>The minimum amount of extracts, add 1ml pyridine an 1ml sodium nitropuside | Pink to red colour appears | Presence of Glycosides |

| S.No | Test | Observation | Inference |
|---|---|---|---|
| 2. | **Keller-killiani test**<br>The minimum amount of extracts, add glacial acetic acid, one drop 5% $FeCl_3$ and Conc. $H_2SO_4$ | Reddish brown colour appears at the junction of the two liquid layers and upper layer appears bluish green | Presence of Glycosies |
| 3. | **Foam test**<br>The minimum amount of extracts was shaken vigorously | Persistent foam was observed | Presence of Glycosies |
| **Test for Tannins and Phenolic Compounds** | | | |
| 1. | The minimum amount of extracts, add 5% $FeCl_3$ | Deep blue-black colour | Tannins/Phenolic compounds |
| 2. | The minimum amount of extracts, add bromine water | Discoloration of bromine water | Tannins/Phenolic compounds |
| 3. | The minimum amount of extracts, add potassium dichromate | Red precipitate | Tannins/Phenolic compounds |
| **Test for Triterpenoids** | | | |
| 1. | The minimum amount of extracts, add little amount of tin and thionyl chloride. | Light red colour solution was obtained | Presence of Triterpenoids |

## Analytical Determination

### FTIR -Fourier Transform Infrared Spectrophotometer[100]

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in the compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

Dried powder of extracts of plant material were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The powdered sample extract of plant specimen was loaded in FTIR spectroscope (Perkine – Elemer FTIR 2000 spectrophotometer), with scan range from 400 $cm^{-1}$ to 4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$

### HPTLC analysis[101]

HPTLC analysis was carried out following Harborne and Wagner et al. For the present study CAMAG HPTLC system equipped with LinomatV applicator, TLC scanner 3 and reprostar 3 controlled by WinCATS-4 software were used. A total of 102 mg extract was dissolved in 10 ml of methanol (95%) and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution.

The samples (5and 10µl) were spotted with a 100 µl Hamilton syringe on a pre-coated silica gel glass plate 60 F 254(20 x 10 cm) (E. Merck) of uniform thickness 0.2mm with aluminium sheet support using a Camag Linomat V. The plates were pre-washed by ethanol and activated at 60° C for 5 min pror to chromatography. The sample loaded plate was kept in Camag TLC glass twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase and the plate was developed up to 90mm in the respective mobile phase.

The Toluene: Ethyl Acetate: Formic acid (5:4:1) was employed as mobile phase. Linear ascending development was carried out in 20 cm x 10cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature [(25±2) °C]. The developed plate was dried at room temperature in air to evaporate solvents from the plate.

The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under White light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag)

**Animal Experimentation**

Pharmacological evaluation of the seaweed extracts was carried out in the Department of Pharmacology, Periyar college of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu, India. Animal facility of this institute is approved by CPCSEA. The experimental protocols for the antigoiterogenic and antiulcer activity activities have been approved by the Insititutional Animal Ethics Committee and conducted according to the guidelines of Indian National Sciences Academy for the use and care of experimental animals. IAEC approved this proposal with approval number PCP/IAEC/001/2017. The animals were maintained at a well ventilated, temperature controlled $30^{o}C \pm 1^{o}C$ animal room for 7days prior to the experimental period and provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before the test. Each animal was used only once.

## Toxicity Studies

### Acute Toxicity Studies[102]

Acute Toxic Class method: Guideline number- 423

The test substance will be administered orally to a group of experimental animals at one of the defined doses. The substance will be tested using a stepwise procedure, each step using three animals of a single sex (normally female). Absence or presence of compound related mortality of the animals dosed at one step will determine the next step i.e.

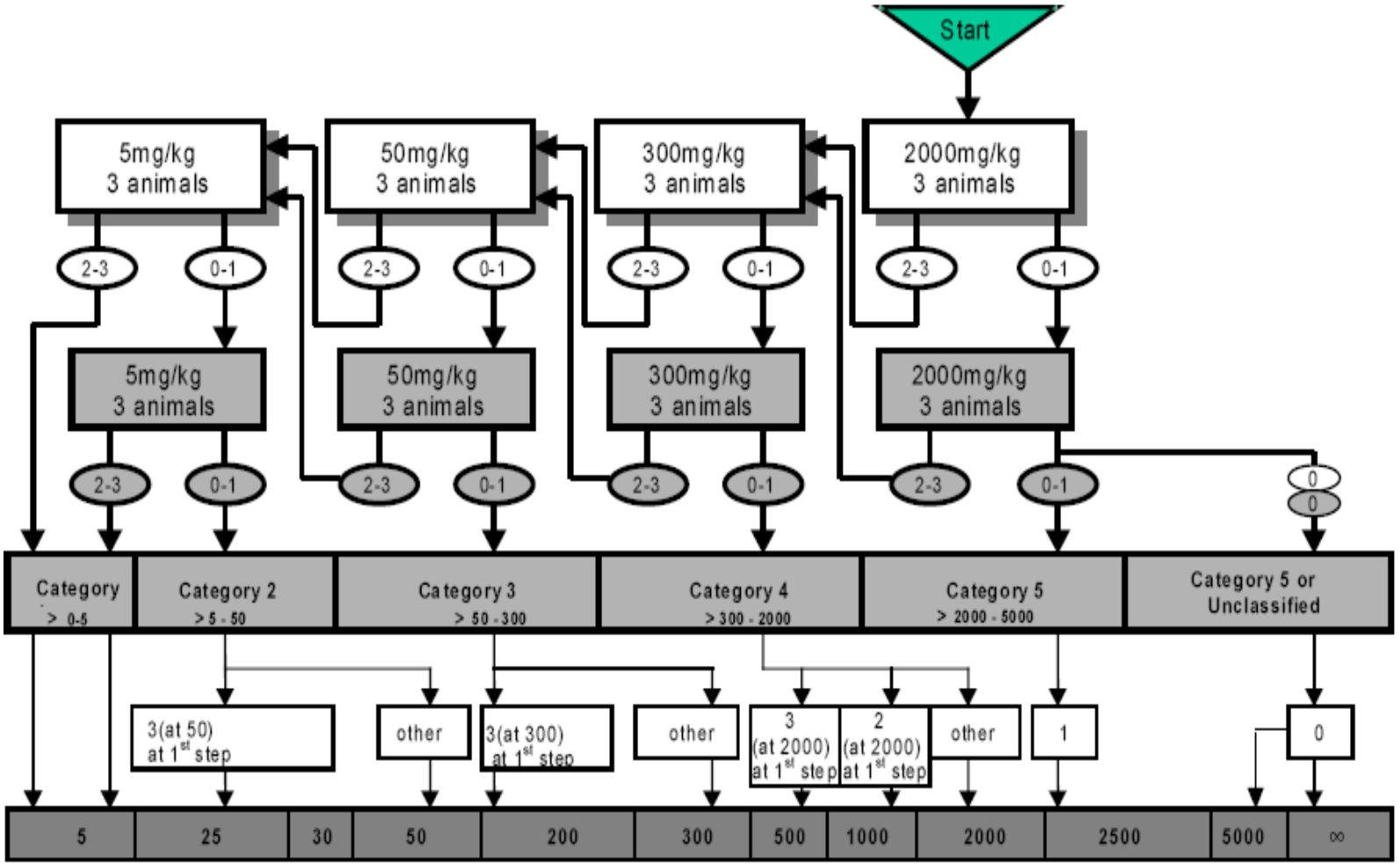
- no further testing is needed
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level

Healthy young mice were used. The test substance was administered in a single dose by gavage using a stomach tube. The dose level to be used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

The starting dose level was most likely to produce mortality in some of the dosed animals. The time interval between treatment groups was determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose was delayed until one is confident of survival of the previously dosed animals. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Observed changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. In addition, behavioral changes, histopathological studies were also observed

**Fig. No. 5: Guideline 423 for acute oral toxicity**



## Anti Goiterogenic Activity[103]

**Anti goiterogenic activity in rats**

Male wistar albino rats weighing 150-200g were divided into four groups of four animals each. The dosage of the drugs were administered to the groups as follows

**Group 1** : Control (saline 0.5ml/kg, *p.o.*)

**Group 2** : Positive control (Propylthiouracil 0.1% *p.o.*)

**Group 3** : Propyl thiouracil + Test drug (Ethanolic extract of *Ulva reticulata,*100mg/kg, *p.o.*)

**Group 4** : Propyl thiouracil + Test drug (Ethanolic extract of *Ulva reticulata*, 200mg/kg, *p.o.*)

**Group 5** : Propyl thiouracil + Standard drug (Thyroxin 10mg/kg, *p.o.*)

The group I served as control and treated with normal saline (0.5ml/kg, *p.o.*). The group II was treated with propyl thiouracil (0.1%, *p.o.*). Group III was treated with propyl thiouracil and ethanolic extract of *ulva reticulata (*100mg/kg, *p.o.*). Group IV was treated with propyl thiouracil and ethanolic extract of *ulva reticulata (*200mg/kg, *p.o.*). The group V received propyl thiouracil and standard drug, thyroxine (10mg/kg, *p.o.*). Goiter was induced by administering 0.1% Propyl thiouracil (PTU) in food or drinking water for a period of 14 days. Drug treatment was started on $15^{th}$ day and continued till $28^{th}$ day. On $28^{th}$ day blood was withdrawn through retero-orbital vein puncture of all groups and the biochemical parameters such as, $T_3$, $T_4$ and TSH were analysed. The thyroid glands were dissected out, weighed rapidly to avoid erratic results. In addition histopathological findings were also performed. The transverse section of the thyroid gland were prepared and viewed in photomicroscopy with 40X magnification.

## Antiulcer Activity [104,105,106]

### Alcohol Induced Ulcer in Albino rats

Adult Wistar albino rats weighing 200-220gm were used for the study. In the laboratory, rats were fed with standard rat pellet diet (Lipton India Ltd, Bangalore) and water *ad libitum.* They were housed in Tarson's polypropylene cages with metal grill tops and acclimated to the laboratory conditions.

Group-I : 1% normal saline (1ml/ 100gm, *p.o.)*

Group-II : Alcohol (1ml/ 200gm, *p.o.)*

Group-III : Lansaprazole (8mg/ kg , *p.o.)*

Group-IV : Ethanolic Extract of *Ulva reticulata* (100mg/ kg , *p.o.*)

Group-V : Ethanolic Extract of *Ulva reticulata* (200mg/ kg , *p.o.*)

The animals are fasted for 24 hours with free access to water. Animals were divided into 5 groups containing four animals in each.The first group of animals were administered 1% normal saline 1ml/100gm, *p.o.*which will serve as negative control. Group II animals were treated with alcohol, 1ml/200gm, *p.o.*which served as positive control. Group III animals were treated with Lansaprazole 8mg/kg, *p.o.*which served as positive control. Group IV animals were treated with Ethanolic Extract of *Ulva reticulate (*100mg/kg , *p.o.).* GroupV Ethanolic Extract of *Ulva reticulata (*200mg/kg, *p.o.)* 1 hr later 1ml/200gm of alcohol (0.1N HCl and 80% ethanol) was administered *p.o.* to each animal.

Animals was sacrificed 1 hr after alcohol administration, stomachs were isolated and cut open along the greater curvature and pinned on the soft board. The ulcer index was measured. The number of ulcers of was noted and severity of ulcers were scored with help of hand lens.

### Statistical analysis

Data are presented as Mean ± SEM. The data was analysed using one way analysis of variance (ANOVA). The statistical significance of the difference of the means was evaluated by Dunnett's multiple comparision test.

## Anti- Microbial Screening[107]

The present investigation was carried out to investigate the chemical and therapeutically potential by evaluating antibacterial and antifungal profile of the Seaweed evaluated

### Determination of Zone of Inhibition

The paper disc diffusion method was used to determine the antimicrobial activities of the newly synthesized compounds. Muller Hington Agar media was prepared, sterilized and used as the growth medium for bacteria culture. 20 mL of the sterilized medium was poured into each sterilized petri dish, covered and allowed to solidify. The plates were then seeded with the test organism (bacterial culture) by sterile cotton swabs

For fungal culture Saboraud Dextrose Agar was prepared and transferred into sterile petri plates and solidified. The sterilised paper discs were soaked in prepared solution of synthesized compounds and were dried at 50ºC. Te dried paper disc was then placed on both plates (Muller Hington and Saboraud Dextrose Agar) seeded with test microorganisms.

The plates were then incubated for bacterial culture for 37 ºC for 24 hours and for fungus the plates were incubated at room temperature for 48 hours and the zone of inhibition were measured

### Organisms Used

### Bacteria

### Gram- positive

- *Staphylococcus aureus* (NCIM 2079)
- *Bacillus substilis* (NCIM 2063)

### Gram- negative

- *Proteus vulgaris* (NCIM 2065)
- *Klebsiella aerogenes* (NCIM 2098)

**Fungi**

- *Aspergillus niger* (NCIM 105)
- *Candida albicans* (NCIM 3102)

**Antibiotic disc used**

- Ciprofloxacin (5µg/ disc) - bacteria
- Nystatin (100 units/ disc) - fungi

## Anti – Oxidant Activity - *In vitro*[108]

**DPPH Scavenging Activity**

DPPH is a stable free radical that can accept an electron or hydrogen to become a stable diamagnetic molecule. Due to its odd electron, the methonolic solution of DPPH shows a strong absorption band at 517nm. DPPH radical reacts with various electron donating molecules (reducing agents or antioxidants). When electrons become paired off, bleaching of the DPPH solution is the result. This result in the formation of the colourless 2, 2' – diphenyl-1-picryl hydrazine. Reduction of the DPPH radicals can be estimated quantitatively by measuring the decrease in absorbance at 517nm.

**Procedure**

Equal volumes of 100µM 2,2' – diphenyl-1- picrylhydrazyl (DPPH) in methanol was added to different concentrations of test compounds (0 - 200µM/mL) in methanol, mixed well and kept in dark for 20 mins.

The absorbance at 517nm was measured using the spectrophotometer, plotting the percentage DPPH. Scavenging against concentration gave the standard curve and the percentage scavenging was calculated from the following equation:

$$\% \text{ Scavenging} = \frac{\text{Absorbance of Blank} \times \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

## 6. RESULTS

### Preliminary Phytochemical Screening

As a part of the preclinical study, the ethanolic extract of *Ulva reticulata* was subjected to qualitative chemical test and confirmed the presence of carbohydrates, alkaloids, steroids, starch, and Proteins compounds shown in the following table

**Table No. 3: Phytochemical Screening**

| S.No | Plant Constituents | Test / reagent | Observation |
|---|---|---|---|
| 1 | Steroids | Salkovaski test | + |
| 2 | Alkaloids | Dragendroff's test<br>Hager's test<br>Mayer's test<br>Wagner's test | + |
| 3 | Saponin | Forms test<br>Haemolysis test | - |
| 4 | Fat and oils | Filter paper test | - |
| 5 | Tanins and phenolic compounds | Ferric chloride test<br>Lead acetate test<br>Pot. Dichromate<br>Bromine water | - |
| 6 | Flavonoids | Shinoda test<br>Lead acetate test | + |
| 7 | Carbohydrates | Molisch test<br>Fehling's test<br>Barfoed's test | + |
| 8 | Proteins | Millons test<br>Biuret test | + |
| 9 | Amnio acid test | Ninhydrine test | - |

(+) = Positive (-) = Negative

## Analytical Determination

### Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The ethanolic extract of *Ulva reticulata* was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of *Ulva reticulata* FTIR analysis confirmed the presence Sterols showed in the **(Table no. 4)**

**Fig. No. 6: FTIR interpretation of EEUR**

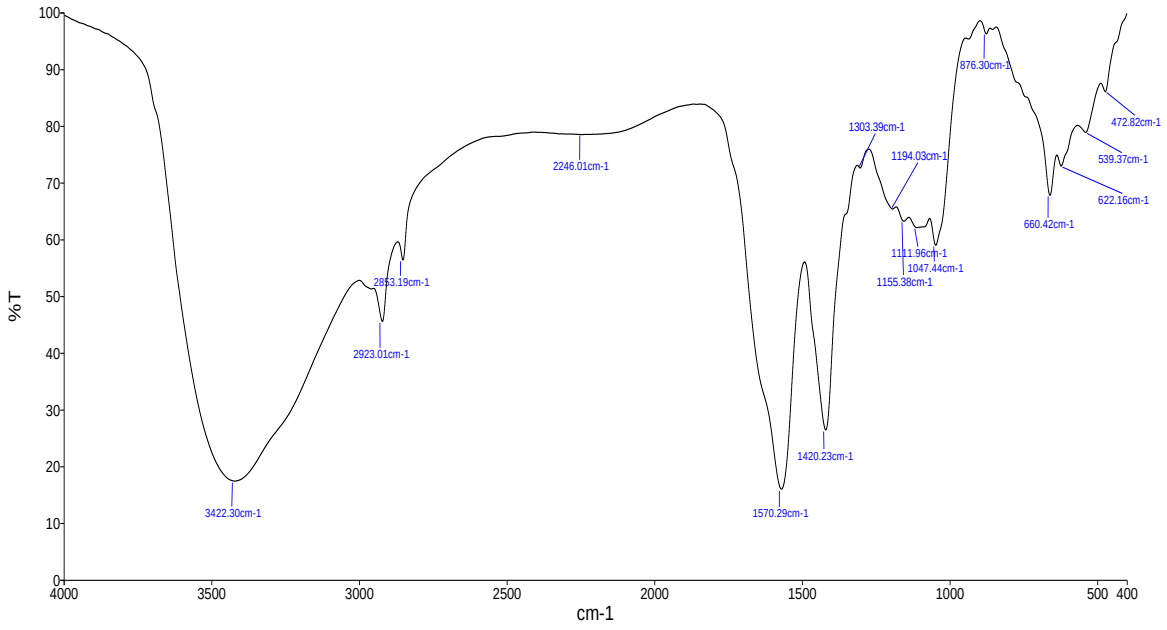


**Table No. 4: FTIR Peak Values and Functional groups of EEUR**

| S.No | Wave Number ($cm^{-1}$) | Functional Group |
|---|---|---|
| 1 | 3422.30 | Secondary amine |
| 2 | 2853.19 | Aldehyde,C-H stretching |
| 3 | 2923.01 | C-H Stretching |
| 4 | 2246.01 | C-H Multiple bond |
| 5 | 1570.29 | Carboxylic acids |
| 6 | 1420.23 | C-H bending |
| 7 | 1303.39 | Unsaturated nitrogen compound |
| 8 | 1111.96 | C-N vibrations |
| 9 | 1047.44 | C-N vibrations aromatic |
| 10 | 1155.38 | C-N vibration |
| 11 | 1194.03 | Unsaturated nitrogen compound |

## HPTLC analysis

The chromatograms shown in **Fig. No. 7** indicate that the sample constituents were clearly separated without any tailing and diffusenses. The investigation of the intense bands obtained from HPTLC profile showed unknown compounds predominantly as shown in **Tables 5 and 6.** A peak display at 5 µl **(Fig. No. 9),** 10 µl **(Fig. No.10)** and a 3D display **(Fig. No. 8)** of the *Ulva* were shown. The peak corresponding to $R_f$ value of 0.67 depicts that it may be due to the presence of Sterols

**Optimized Chromatographic conditions**

Stationary Phase : Merck HPTLC plates coated with Silica Gel 60 $F_{254}$ of 0.2 mm thickness

Mobile Phase : Toulene: Ethyl Acetate: Formic acid (5:4:1)

Sample Applicator : Camag linomat V automatic applicator

Scanner : Camag Scanner

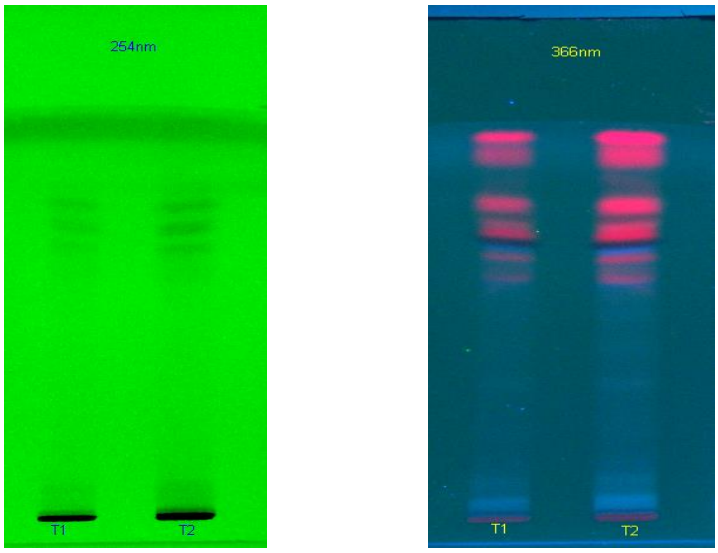
Syringe : Hamilton syringe (100 µl)

Wavelength : 254 nm

## HPTLC fingerprinting profile Ethanolic extract of *Ulva reticulata*

**Fig. No. 7: Photo Documentation Under UV**

**AT 254nm** **AT 366nm**




T1 T2 T1 T2

**TLC details**

Track T1-5µl of Ethanolic Exract

Track T2-10 µl of Ethanolic Exract

**Fig. No. 8: Chromatogram 3D display at 254 nm**

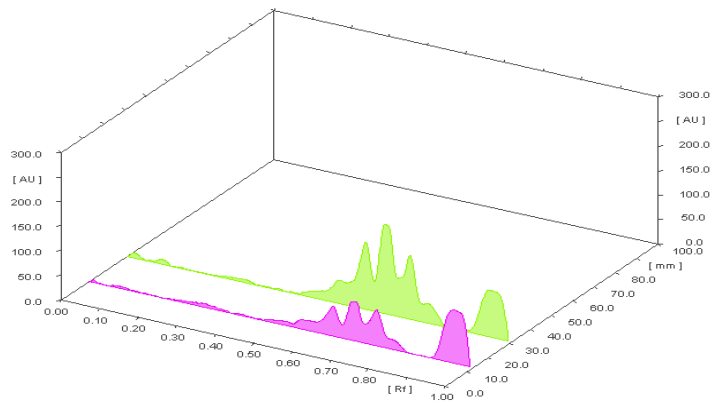


**Fig. No. 9: Peak Display (5µl of test solution)**

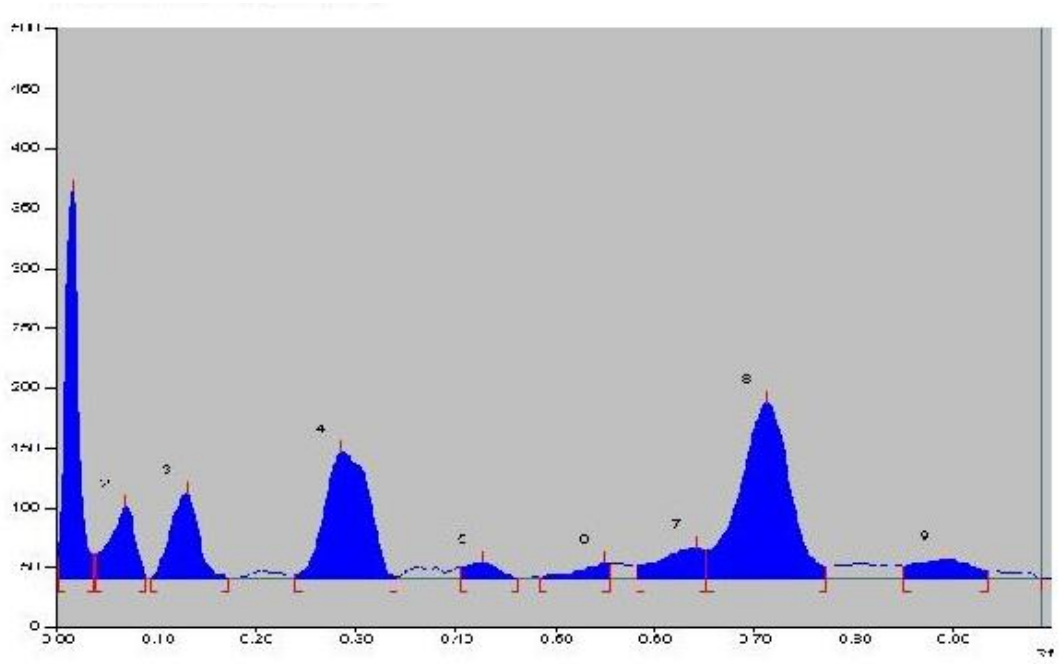



**Table No. 5: Chromatogram Data for (5µl of test solution)**

| Peak | Start $R_f$ | Start Height | Max $R_f$ | Max Height | Height % | End $R_f$ | End Height | Area | Area % | Assigned Substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.53 | 6.7 | 0.56 | 19.6 | 5.78 | 0.56 | 19.2 | 344.9 | 3.06 | Unknown* |
| 2 | 0.59 | 20.4 | 0.63 | 60.2 | 17.71 | 0.66 | 27.1 | 1766.1 | 15.66 | Unknown* |
| 3 | 0.66 | 27.5 | 0.69 | 79.3 | 23.33 | 0.72 | 38.9 | 2366.5 | 20.98 | Unknown* |
| 4 | 0.72 | 39.1 | 0.75 | 72.9 | 21.45 | 0.84 | 0.2 | 2333.2 | 20.69 | Unknown* |
| 5 | 0.88 | 0.1 | 0.95 | 107.8 | 31.74 | 0.99 | 34.8 | 4466.0 | 39.61 | Unknown* |

**Fig. No. 10: Peak Display (10µl of test solution)**

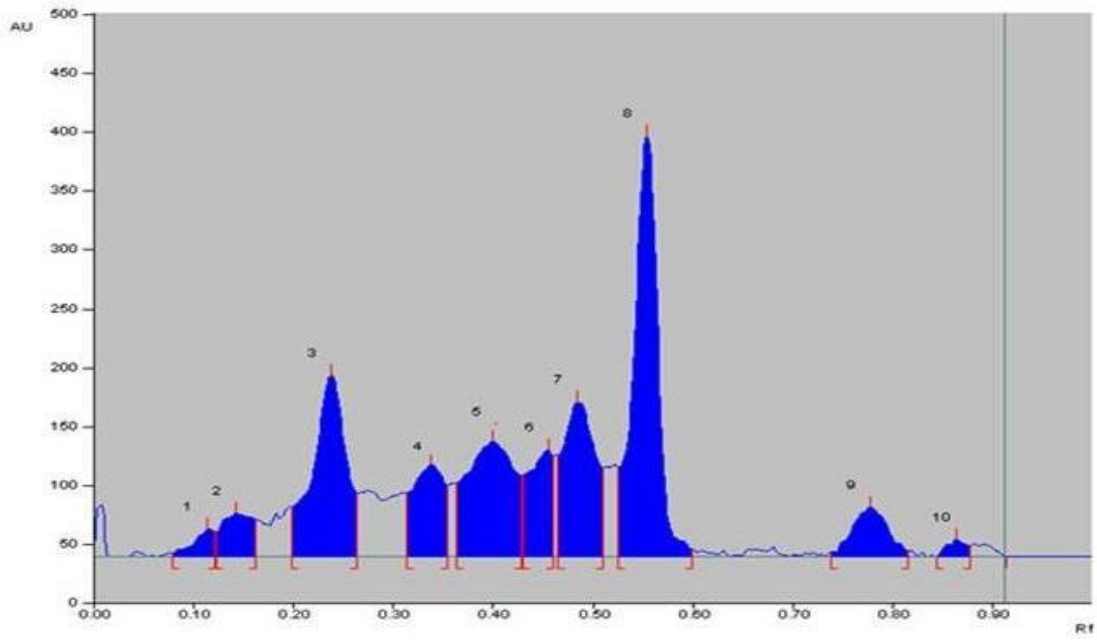



**Table No. 6: Chromatogram Data for (10µl of test solution)**

| Peak | Start $R_f$ | Start Height | Max $R_f$ | Max Height | Height % | End $R_f$ | End Height | Area | Area % | Assigned Substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.0 | 0.1 | 0.02 | 11.0 | 1.34 | 0.04 | 1.7 | 139.3 | 0.44 | Unknown* |
| 2 | 0.10 | 10.8 | 0.17 | 188.6 | 22.86 | 0.22 | 2.6 | 5891.9 | 18.06 | Unknown* |
| 3 | 0.27 | 4.7 | 0.37 | 72.7 | 8.80 | 0.41 | 23.2 | 2654.3 | 8.42 | Unknown* |
| 4 | 0.41 | 23.3 | 0.48 | 64.5 | 7.81 | 0.51 | 50.6 | 2880.6 | 9.14 | Unknown* |
| 5 | 0.51 | 50.7 | 0.56 | 73.0 | 8.85 | 0.58 | 65.4 | 2960.1 | 9.39 | Unknown* |
| 6 | 0.59 | 65.4 | 0.63 | 116.5 | 14.11 | 0.67 | 60.0 | 4499.2 | 14.27 | Unknown* |
| 7 | 0.67 | 60.1 | 0.74 | 87.8 | 10.64 | 0.77 | 66.1 | 4629.3 | 14.69 | Unknown* |

## Pharmacological Evaluation

### Acute Oral Toxicity

**Table No. 7: Behavioral Changes in Acute Oral Toxicity**

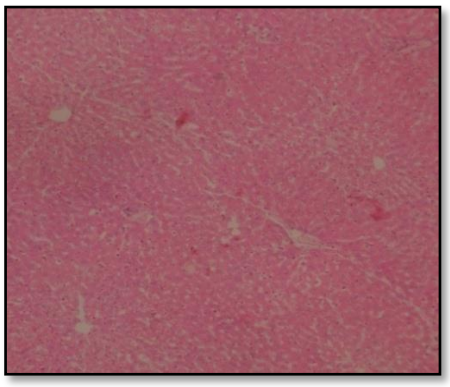
| S.No. | Symptoms | Ethanolic extract of *Ulva reticulate* (2000mg/kg.p.o) |
|---|---|---|
| 1 | Death | -- |
| **Autonomous Nervous System** | | |
| 2 | Head movements | -- |
| 3 | Scratching | ++ |
| 4 | Altered reactivity to touch | -- |
| 5 | Loss of righting reflex | -- |
| 6 | Loss of corneal reflex | -- |
| 7 | Defecation/Diarrhea | -- |
| 8 | Salivation | -- |
| 9 | Lacrimation | -- |
| 10 | Myosis/ Mydriasis | -- |
| 11 | Loss of traction | -- |
| **Central Nervous System** | | |
| 12 | Convulsions | -- |
| 13 | Tremor | -- |
| 14 | Straub tail | -- |
| 15 | Sedation | -- |
| 16 | Excitation | ++ |

| S.No. | Symptoms | Ethanolic extract of *Ulva reticulate* (2000mg/kg.p.o) |
|---|---|---|
| 17 | Jumping | -- |
| 18 | Abnormal gait | -- |
| 19 | Motor in-coordination | -- |
| 20 | Altered muscle tone | -- |
| 21 | Akinesia | -- |
| 22 | Catalepsy | -- |
| 23 | Loss of balance | -- |
| 24 | Fore-paw treading | -- |
| 25 | Writhing | -- |
| 26 | Stereotypy | -- |
| 27 | Altered fear | ++ |
| 28 | Altered respiration | -- |
| 29 | Aggression | ++ |
| 30 | Analgesia | -- |
| 31 | Body Temperature | -- |

**Histopathological Studies**

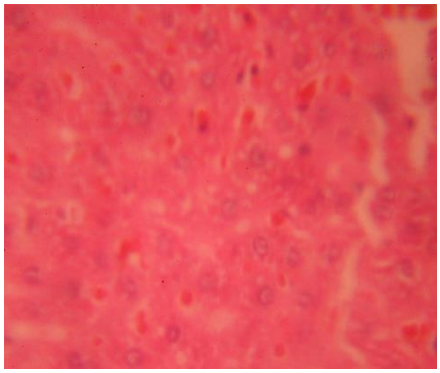
**T.S of Liver**

**Fig No. 11**



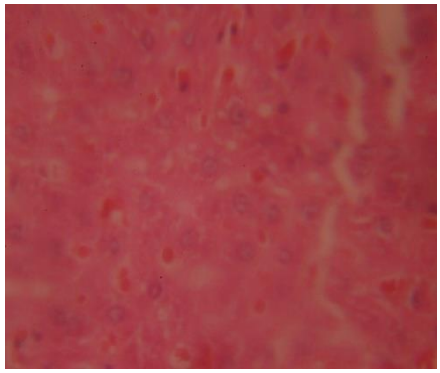
Control **(**Normal saline 5ml/kg)

**Fig No. 12**



(Ethanolic extract of *Ulva reticulata* 100mg/kg *p.o.*)

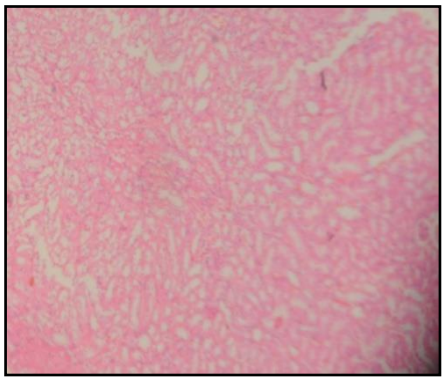
**Fig No. 13**



(Ethanolic extract of *Ulva reticulata* 200mg/kg *p.o.*)

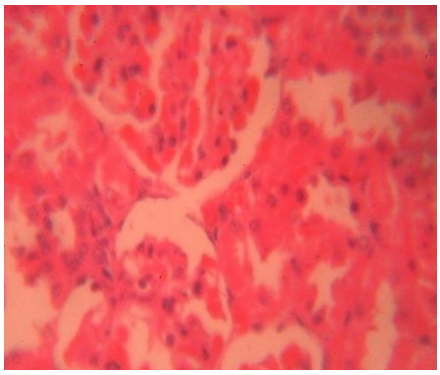
**T.S. of Kidney**

**Fig. No. 14**



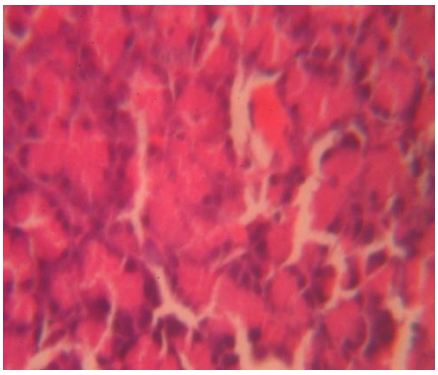
Control **(**Normal saline 5ml/kg)

**Fig No. 15**



(Ethanolic extract of *Ulva reticulata* 100mg/kg *p.o*)

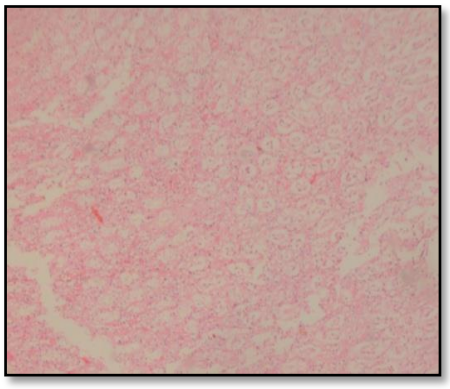
**Fig. No. 16**



(Ethanolic extract of *Ulva reticulata* 200mg/kg *p.o*)

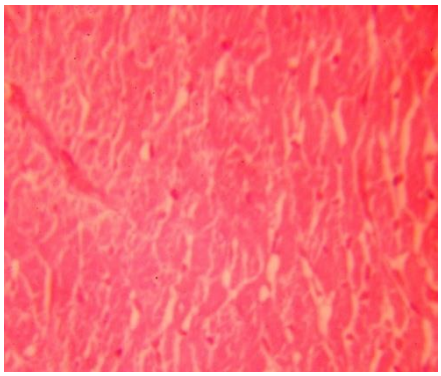
**T.S. of Heart**

**Fig. No. 17**



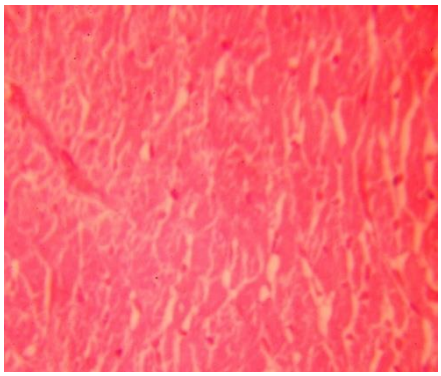
Control **(**Normal saline 5ml/kg)

**Fig. No. 18**



(Ethanolic extract of *Ulva reticulata* 100mg/kg *p.o*)

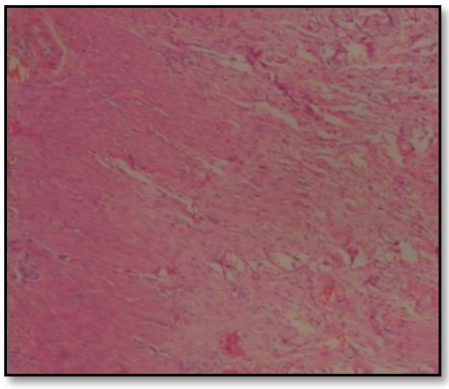
**Fig. No. 19**



(Ethanolic extract of *Ulva reticulata* 200mg/kg *p.o*)

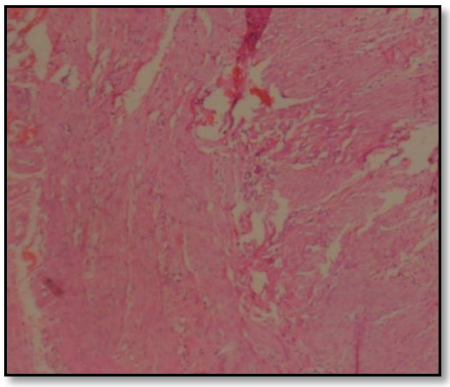
**T.S. of Pancreas**

**Fig. No. 20**



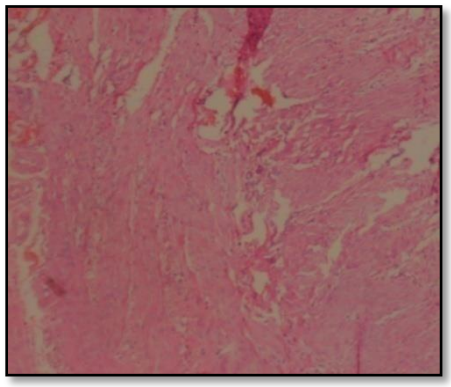
Control **(**Normal saline 5ml/kg)

**Fig. No. 21**



(Ethanolic extract of *Ulva reticulata* 100mg/kg *p.o*)

**Fig. No. 22**



(Ethanolic extract of *Ulva reticulata* 200mg/kg *p.o*)

**Table No. 8: Histopathology Studies of Acute Oral Toxicity**

| S.no | Treatment | Liver | Kidney | Heart | Pancreas |
|---|---|---|---|---|---|
| 1 | Control | Normal architecture | Normal architecture | Normal architecture | Normal architecture |
| 2 | Ethanolic extract of *Ulva reticulata* 100mg/kg *p.o*) | No fatty deposition and necrosis of hepatic cells | No damage found in the glomerulus cells | No change in the pattern of myocardial cells | Islets of langerhans seems normal |
| 3 | Ethanolic extract of *Ulva reticulata* 200mg/kg *p.o*) | Normal | Normal architecture | No damage | No distinguished damage to cells of islets of langerhans |

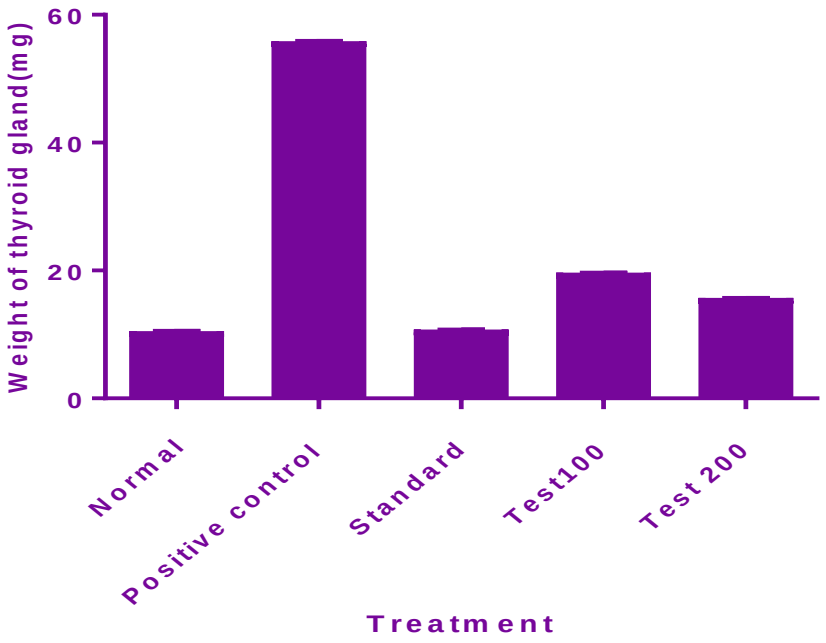
**Table No. 9: Effect of ethanolic extract of *Ulva reticulata* on changes in thyroid gland weight in propylthiouracil induced goiterogenic rats**

| S.No. | Treatment | Thyroid gland weight (mg) |
|---|---|---|
| 1 | Normal (normal saline, 0.5ml / kg, *p.o.*) | 10.55 ± 0.161 |
| 2 | Positive control (Propyl thiouracil, 0.1% *p.o*) | 55.883 ± 0.730 |
| 3 | Test (Ethanolic extract of *Ulva reticulata*, 100mg/kg *p.o.*) | 19.65 ± 0.01 |
| 4 | Test (Ethanolic extract of *Ulva reticulata*, 200mg/kg *p.o.*) | 15.717 ± 0.399*** |
| 5 | Standard (Thyroxin 10mg/kg *p.o.*) | 10.750 ± 0.320*** |

**n = 4 values are expressed as ± S.E.M.**

***** P < 0.0001 Vs Control by one way ANOVA**

**Fig. No. 23: Effect of ethanolic extract of *Ulva reticulata* on changes in thyroid gland weight in Propyl thiouracil induced goiterogenic rats**




Normal - (normal saline, 0.5ml / kg, *p.o.*)

Positive control - (Propyl thiouracil, 0.1% *p.o.*)

Test 1 - (Ethanolic extract of *Ulva reticulata*, 100mg/kg *p.o.*)

Test 2 - (Ethanolic extract of *Ulva reticulata*, 200mg/kg *p.o.*)

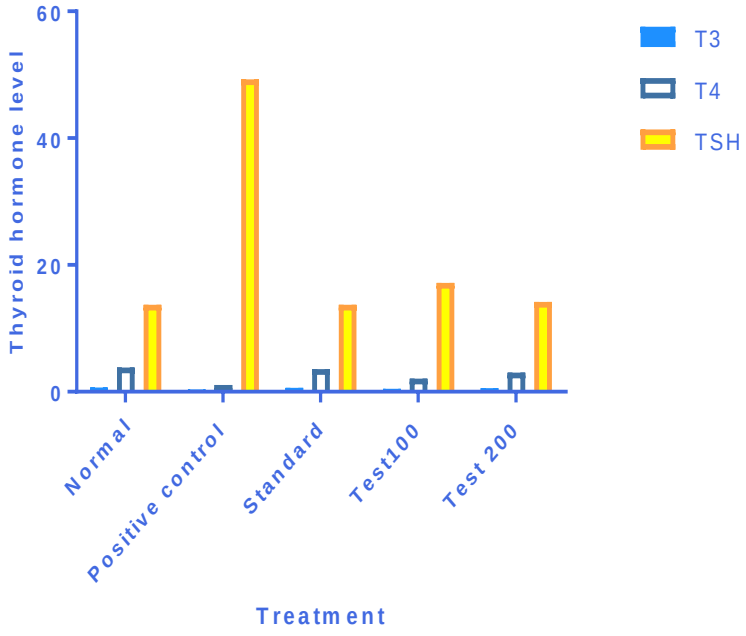
**Table No. 10: Effect of ethanolic extract of *Ulva reticulata* on thyroid hormone levels in Propyl thiouracil induced goiterogenic rats**

| S.No | Treatment | T3 (ng/ml) | T4(µg/dl) | TSH (µIU/ml) |
|---|---|---|---|---|
| **1** | **Normal** (normal saline, 0.5ml / kg, *p.o.*) | 0.622 ± 0.005 | 3.833 ± 0.049 | 13.733 ± 0.049 |
| **2** | **Positive control** (Propyl thiouracil, 0.1% *p.o.*) | 0.373 ± 0.009 | 1.08 ± 0.03 | 49.272 ± 0.306 |
| **3** | **Test I** (Ethanolic extract of *Ulva reticulata*, 100mg/kg *p.o.*) | 0.48 ± 0.03 | 2.045 ± 0.03 | 17.16 ± 0.005 |
| **4** | **Test II** (Ethanolic extract of *Ulva reticulata*, 200mg/kg *p.o.*) | 0.565 ± 0.011*** | 3.035 ± 0.068*** | 14.15 ± 0.322*** |
| **5** | **Standard** (Thyroxin 10mg/kg *p.o.*) | 0.623 ± 0.011*** | 3.588 ± 0.032*** | 13.733 ± 0.176*** |

**n = 4 values are expressed as ± S.E.M**

***** P < 0.0001 Vs Control by one way ANOVA**

**Fig. No. 24: Effect of ethanolic extract of *Ulva reticulata* on thyroid hormone levels in Propyl thiouracil induced goiterogenic rats**




Normal - (normal saline, 0.5ml / kg, *p.o.*)

Positive control - (propyl thiouracil, 0.1% *p.o.*)

Standard - (Thyroxin 10mg/kg *p.o.*)

Test 1 - (Ethanolic extract of *Ulva reticulata*, 100mg/kg *p.o.*)

Test 2 - (Ethanolic extract of *Ulva reticulata*, 200mg/kg *p.o.*)

**Microscopic investigation of T.S of thyroid gland in goiterogenic rats**

**Fig. No. 25: T. S of Thyroid gland Propyl thiouracil treated rat**

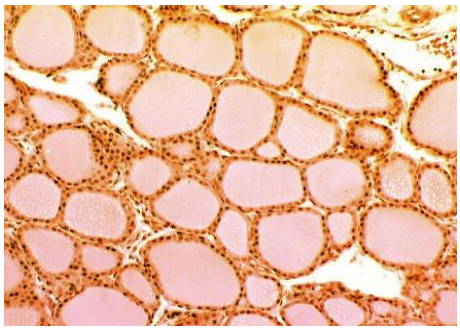


**Fig. No. 26: T. S of Thyroid gland Ethanolic extract of *Ulva reticulata* 100mg/kg**

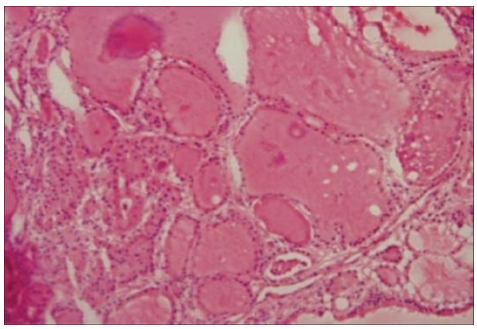


**Fig. No. 27: T.S of Thyroid gland Ethanolic extract of *Ulva reticulata* 200mg/kg**

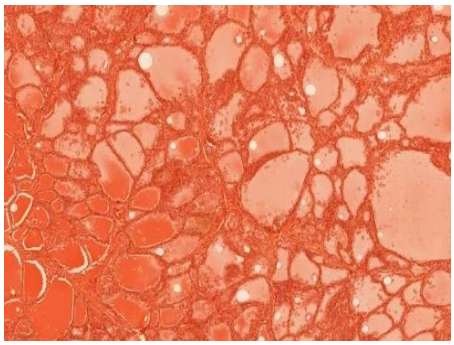


**Fig. No. 28: T.S of Thyroid gland Thyroxine treated in rat**

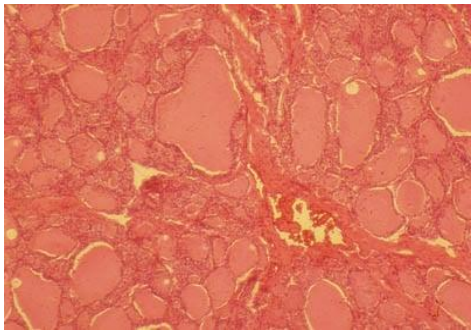


**Table No. 11: Microscopic investigation of T.S of thyroid gland in goiterogenic rats**

| S.No | Parameters | Goiter control (propyl thiouracil, 0.1% p.o) | Test I (Ethanolic extract of *ulva reticulata*, 100mg/kg p.o.) | Test II (Ethanolic extract of *ulva reticulata*, 200mg/kg p.o.) | Standard (Thyroxin 10mg/kg p.o.) |
|---|---|---|---|---|---|
| **1** | Grey white soft tissue | Irregular | Regular | Regular | Regular |
| **2** | Cellular debris | Present | Less Cellular debris | Very Less Cellular debris | No Cellular debris |
| **3** | Grey – brown soft tissue | Irregular | Regular | Regular | Regular |
| **4** | Colloid formation | Nil | Yes | Yes | Yes |
| **5** | Follicles packing | Irregular | Closed packing | Closed packing | Closed packing |

**Table.12: Effect of EEUR on Alcohol induced ulcer in Rats**

| Treatment | Ulcer Index | | | | | Total Score Mean ± SEM | % Inhibition |
|---|---|---|---|---|---|---|---|
| | Red coloured stomach | Spot ulcer | Haemorragh streaks | Ulcers <3mm | Ulcers >3mm | | |
| **Control** (Normal saline), 1ml p.o. | 1 | 1 | - | - | - | 0.333 ± 0.247 | - |
| **Ulcer control** Alcohol (1 ml/ 200 gm, p.o) | 3 | 44 | 36 | - | 6 | 15.167 ± 2.108 | - |
| **Standard** Alcohol (1 ml/ 200 gm,p.o) + Lansaprazole (8mg/kg,p.o) | - | 25*** | 13.5*** | 2 | - | 6.667 ± 0.872** | 55.49*** |
| **Test I** Alcohol (1 ml/ 200 gm,p.o.) + *Ulva reticulata* (100mg/kg, p.o) | - | 26*** | 3*** | 2 | - | 5.16 ± 0.527* | 65.93** |
| **Test II** Alcohol (1 ml/ 200 gm,p.o.) + *Ulva reticulata* (200mg/kg, p.o) | - | 25*** | 1.5*** | 2 | - | 4.417 ± 0.271* | 70.87** |

**n = 4. Total Score Values are expressed as ± S.E.M. ***P < 0.001, ns P > 0.05 Vs Control (One way ANOVA followed by Dunnett's test)**

**Photos of Antiulcer activity of *Ulva reticulata* treated rat in**

**Alcohol induced ulcer model**

**Fig. No. 29**

**Normal**

**Fig. No. 30**

**Ulcer Control**

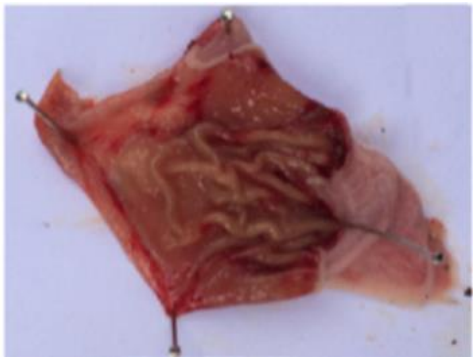


**Fig. No. 31**

**Lansaprazole (8mg/kg)**

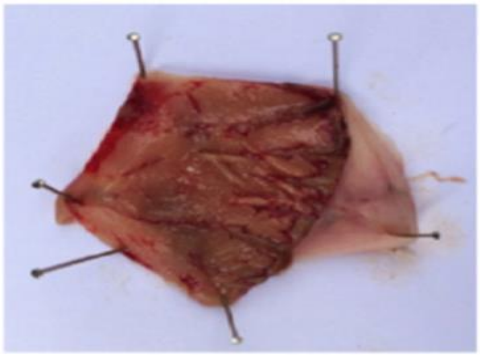


**Fig. No. 32**

***Ulva reticulata* (100mg/kg)**

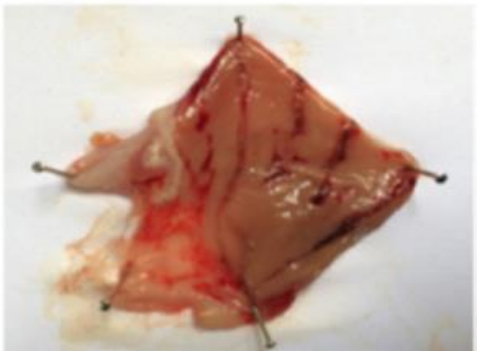


**Fig. No. 33**

***Ulva reticulata* (200mg/kg)**

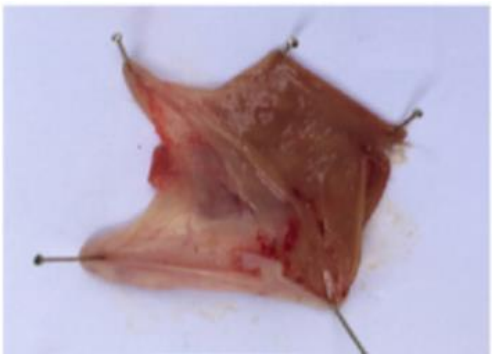
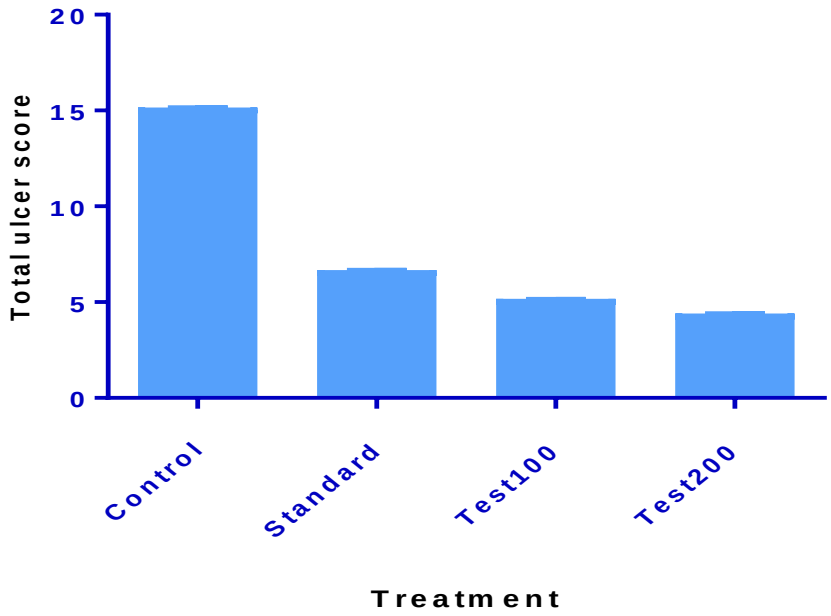


**Table No. 13: Description of Microscopy on Ethanol induced Ulcer**

| S.no | Treatment | Ulcer index |
|---|---|---|
| 1 | Normal | Normal Architecture |
| 2 | Ulcer control | More spot ulcers and haemorrhagic streaks and reduced the ulcers >3mm |
| 3 | Standard Lansaprazole(8mg/kg) | Less spot ulcers and haemorrhagic streaks and reduced the ulcers >3mm |
| *4* | Test I *Ulva reticulata* (100mg/kg) | Reduced the spot ulcers and haemorrhagic streaks and reduced the ulcers >3mm |
| *5* | Test II *Ulva reticulata* (200mg/kg) | More reduction in the spot ulcers and haemorrhagic streaks and reduced the ulcers >3mm |

**Fig. No. 34: Effect of test compound Ethanolic extract *of Ulva reticulata* on Alcohol induced ulcer model in rats**




Ulcer control - Alcohol (1 ml/200 gm, *p.o.*)

Standard - Alcohol (1 ml/ 200 gm, *p.o.*) + Lansaprazole (8mg/kg, *p.o.*)

Test 1 - Alcohol (1 ml/200 gm, *p.o.*) + *Ulva reticulata* (100mg/kg, *p.o.*)

Test 2 - Alcohol (1 ml/200 gm, *p.o.*) + *Ulva reticulata* (200mg/kg, *p.o.*)

**Table No. 14: Antimicrobial activity of test compound EEUR**

| S.No. | Name of the Microorganism | Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Solvent control** | **Standard** | **100 µg** | **75 µg** | **50 µg** | **25 µg** |
| 1. | *Staphylococcus aureus* ( NCIM 2079) | NIL | 35 | 20 | 16 | 12 | 12 |
| 2. | *Bacillus subtilis* (NCIM 2063) | NIL | 40 | 14 | 12 | 09 | 08 |
| 3. | *Proteus vulgaris* (NCIM 2027) | NIL | 30 | 10 | NIL | NIL | NIL |
| 4. | *Klebsiella aerogenes* (NCIM 2098) | NIL | 30 | 18 | 16 | 15 | 13 |
| 5. | *Candida albicans* (NCIM 3102) | NIL | 32 | 12 | 12 | 10 | NIL |
| 6. | *Aspergillus niger* (NCIM 105) | NIL | 35 | 18 | 15 | 10 | 08 |

Standard- Ciprofloxacin 5µg/disc for bacteria; Nystatin 100 units/disc for fungi

Solvent- DMSO (dimethyl sulphoxide)

**Anti - Microbial Activity**

**Anti Bacterial Activity**

**Fig. No. 35: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Proteus vulgaris***


**Fig. No. 36: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Klebsiella aerogenes***

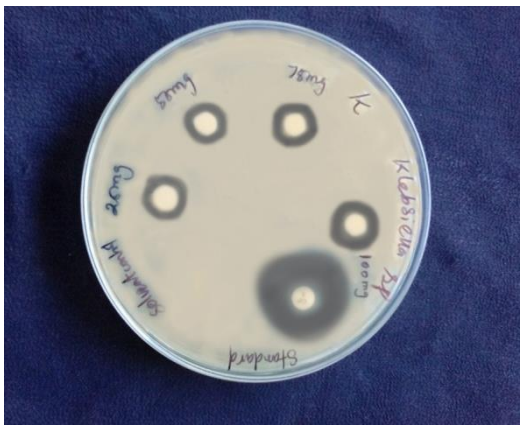


**Fig. No. 37: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Bacillus subtilis***

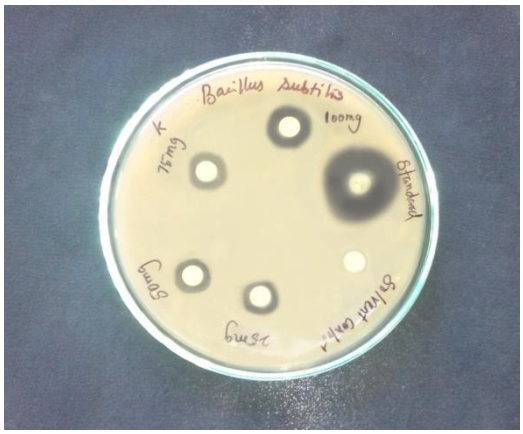
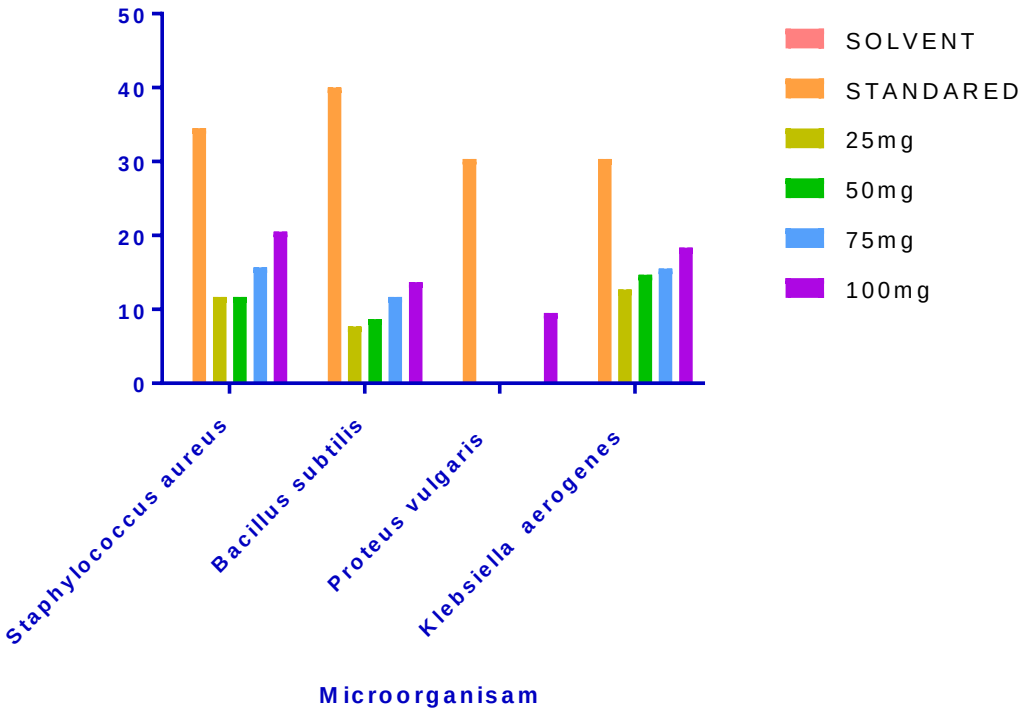



**Fig. No. 38: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Staphylococcus aureus***

**Fig. No. 41: Antimicrobial activity of Test drug (EEUR)**

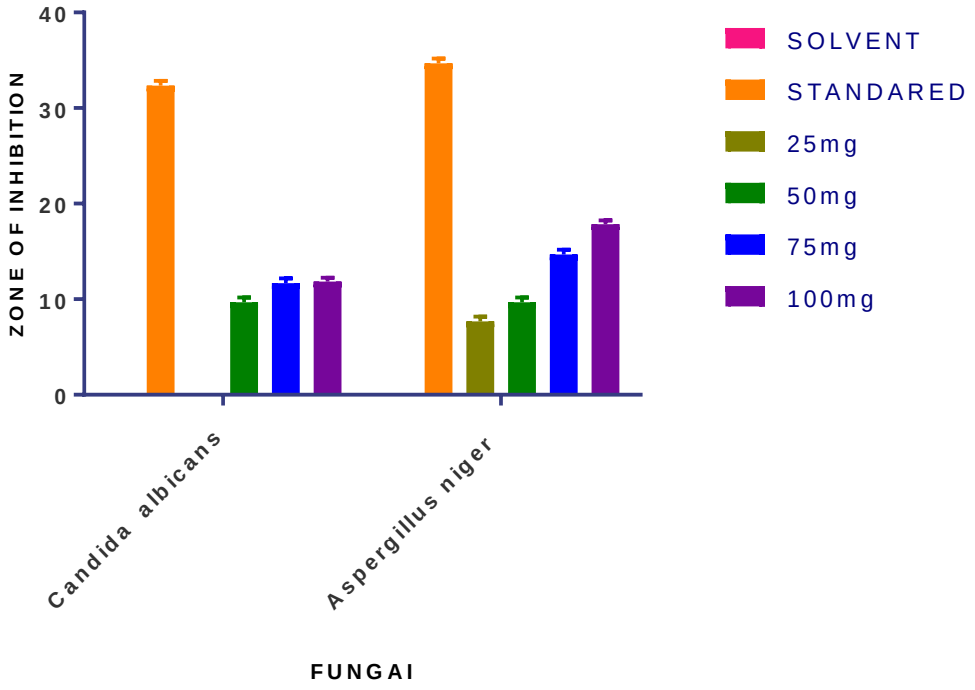


**Anti - Fungal Activity**

**Fig. No. 39: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Aspergillus niger***


**Fig. No. 40: Zone of inhibition of test compound 25µg, 50µg, 75µg and 100µg against *Candida albicans***

**Fig. No. 42: Anti - fungal activity test drug (EEUR)**



**Antioxidant Activity (*in vitro*)**

**Effect of Absorbance on EEUR by DPPH assay**

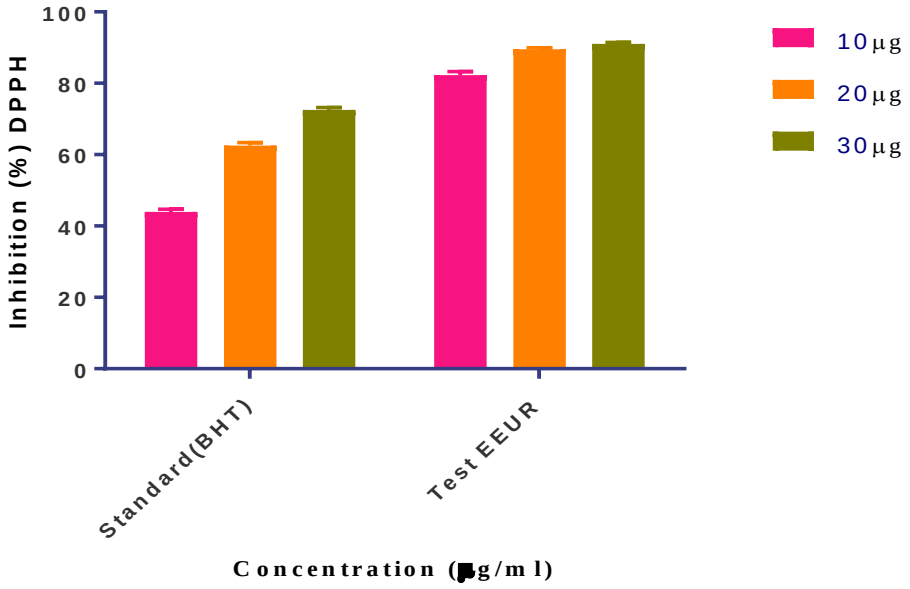
DPPH Radical scavenging assay EEUR at 30µg/ml concentration showed maximum DPPH radical scavenging activity i.e. 91.05% where as BHT at the same concentration exhibited 72.57% inhibitions. The IC50 values were found to be 20 µg/ml and 10 µg/ml EEUR and BHT respectively.

Free radical scavenging capacity of *Ulva reticulata* may be due to the flavonoids,which are typical phenolic compounds,act as metal chelators and Free radical scavengers.

**Table No. 15: Effect of EEUR on DPPH Radical scavenging assay**

| S. No | Name of the sample | % Free radical scavenging activity ($IC_{50}$ values) | | |
|---|---|---|---|---|
| | | Dose | | |
| | | 10µg | 20µg | 30 µg |
| 1 | Standard(BHT) | 43.93 | 62.57 | 72.57 |
| 2 | Test (EEUR) | 83.31 | 89.52 | 91.05 |

**Fig. No. 43: Anti oxidant activity of test drug (EEUR)**




Standard (BHT) – Butylated Hydroxy Toluene

Test - Ethanolic extract *of Ulva reticulata*

## DISCUSSION

### Preliminary phytochemical studies

To The crude extract was subjected to qualitative chemical test to confirm the presence of alkaloids, proteins, carbohydrates, Steroids, and flavanoids in ethanolic extract of *Ulva reticulata* **(Table No.3)**

### Acute oral toxicity

Administration of *Ulva reticulata* at a dose of 2000 mg/kg body weight did not produce any behavioural abnormalities in the animals except scratching, exitation, altered fear and aggression. As all tested animals survived, the oral $LD_{50}$ of *Ulva reticulata* in mice was found to be 200 mg/kg body weight. **(Table No.7, 8)**

### Evaluation of antigoiterogenic activity

The antigoiterogenic effect of *Ulva reticulata* showed decrease in thyroid gland weight summarised in **(Table No. 9).** All the treated groups showed reduction in thyroid gland weight compared with goiterogenic control rats.

The antigoiterogenic effect of ethanolic extract of *Ulva reticulata* in rats was presented in **Table No. 10.** When goiterogenic rats treated with ethanolic extract of *Ulva reticulata* for 14days the $T_3$ and $T_4$ levels were increased and TSH level was decreased. The increased $T_3$ and $T_4$ levels and reduced TSH levels were significant (***$P<0.0001$) when compared with standard thyroxine.

The effects may be due to increase in iodination of tyrosine residues in thyroglobulin and also due to enhanced coupling of iodotyrosine residues to form $T_3$ and $T_4$.

The T.S of thyroid gland showed cubial epithelial cells and follicular lumina filled with colloid in control group. In goiter control rats, it showed enlarged blood vessels, absence of colloid formation and narrow follicular lumen.

Animals treated with ethanolic extract of *Ulva reticulata* showed the section contained regular grey brown soft tissue, grey white soft tissue and regular follicle with colloid filled in their lumina. There was less cellular debris and no necrotic cells in the follicular lumina when compared with standard drug thyroxine **(Table No. 11).**

**Alcohol induced ulcer**

Results of antiulcer activity of *Ulva reticulata* on alcohol induced ulcer are shown in **Fig.32, Fig.33 and Table.12& 13** The results showed significant increase in ulcer score in alcohol control group compared to normal control. The ulcer score decreased after treatment with *uUva reticulata* (***P<0.0001) and lansaprazole there by decreasing the damage to gastric mucosa of stomach and formation of HCl secretion.

Antiulcer activity of *Ulva reticulata* was determined by alcohol induced ulcer. Stomach being the principal organ of ulcer, alcochol administration to the experimental fasting animals 24 hours resulted in various degrees of ulcers.

Alcohol increases the risk of ulcer by damaging the gastric mucosa of the stomach and increasing the gastric HCl of the stomach. The genesis of alcohol induced gastric lesions is multifactorial with the depletion of gastric wall mucous content as one of the involved factors. It is also associated with significant production of free radicals, leading to an increased oxidative stress and damage to the cell and cell membrane. The pepsin may have a role in the etiology of gastric ulceration and cancer. This suggests that inhibitors of acid secretion may prevent ulceration not only by the removal of acid but also by inactivation of pepsin following the subsequent rise in gastric pH. Therefore acid secretion may not have to be suppressed to prevent the development of gastric ulcers since the inhibition of pepsin activity alone may be sufficient to heal the ulcers and the side effects of suppressing acid secretion can be avoided. Proteolytic activity of pepsin as the primary aggressor in gastric mucosal ulceration

**Antimicrobial activity of Test Drug**

The present investigation was carried out to find out the chemical and therapeutic potency of Seaweed by evaluating antibacterial and antifungal profile using Disc Diffusion method and the results are shown in the **Table No: 14** and the **Fig. No: 35 – 40.** The Test Drug possess significant antibacterial activity against the gram positive strains such as *Staphylococcus aureus, Bacillus substilis* and the gram negative strains such as *Proteus vulgaris, Klebsiella aerogenes*. The zone of inhibition was increased for the test compound when compared to the standard (Ciprofloxacin)

The antifungal activity also performed and the results are found that the Test Drug possess significant activity *Candida albicans* by means of the increased zone of inhibition

and the another strain *Aspergillus niger* possess lesser zone of inhibition when compared with that of the standard (Nystatin)

**Antioxidant activity of Test Drug**

The *in vitro* antioxidant activity of test Compound was determined by 2, 2' – diphenyl-1- picrylhydrazyl (DPPH) assay. Results are presented in **Table No: 15** and **Fig. No: 43.** The investigations indicated that the Test Drug was found to have significant effect on free radicals which was well comparable with standard drug Butylated Hydroxyl Toluene (BHT). It was found to exert a beneficial action against peroxidases generated by DPPH assay method with an $IC_{50}$ of 83.31 while BHT showed $IC_{50}$ of 43.93 at 10µg

Test Compound provides satisfactory cytoprotective effect by exhibiting protection against peroxidative changes by imparting cellular membrane stability and involves inhibition of free radical production along with enhancement of the body defence system.

# 7. CONCLUSION

- Phytochemical screening confimed the presence of Steroids, alkaloids, flavanoids, carbohydrates and proteins and revels the scope for Pharmacological activity

- FTIR peak showed the alkanes, ketones, alkyl halides, alkynes are present in ethanolic extract of *Ulva reticulata*

- HPTLC analysis confirmed the presence of sterols in Ethanolic extract of *Ulva reticulata*

- The test compound possess better antioxidant activity and Percentage of free radical scavenging activity is increased when compared with that of the standard ascorbic acid and Butylated Hydroxy Toluene

- Test compound posses antibacterial effects against the gram positive organisms *Bacillus subtilis, Staphylococcus aureus* and gram negative organism *Klebsiella aerogenes & Proteus vulgaris*.The test compounds are having greater effect against the fungal strain *Candida albicans* when compared with that of the standard and the zone of inhibition of another fungal strain *Aspergillus niger* was lesser when compared with that of the standard (Nystatin)

- In view of the obtained results, it has been evident that the ethanolic extract of *Ulva reticulata* has been observed to exert significant and consistent antigoiterogenic effect in propyl thiouracil induced goiter.

- The present study indicating the presence of Ulcerogenic effect in Test compound aspirin induced ulcer models possibly through a direct corrosive effect on gastric epithelium, leading to mucosal damage on the glandular part of the stomach and aspirin induced gastric damage is due to direct irritant effect of aspirin on gastric mucosa, increased acid secretion and decreased mucin secretion due to inhibition of prostaglandins (PG) synthesis. The Test compound are slightly possess ulcerogenic effect when compared with the ulcer control

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